STUDIES IN CHEMISTRY OF O & N HETEROCYCLES AND PLANT PRODUCTS

SUMMARY

THESIS

SUBMITTED FOR THE AWARD OF THE DEGREE OF

Doctor of Philosophy

IN

CHEMISTRY

BY

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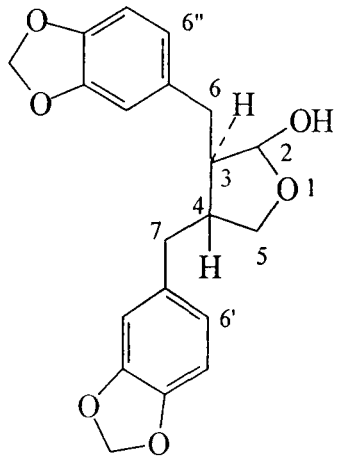
DEPARTMENT OF CHEMISTRY
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)

2006

SUMMARY

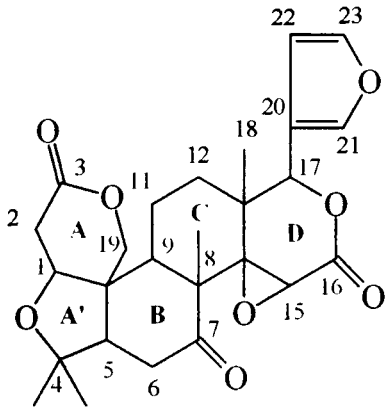
The work presented in the thesis is based in part on investigation of the constituents of the medicinal herb *Piper cubeba* and *Zanthoxylum simularis*. The petrol and benzene extracts of seeds of *Piper cubeba* exhibited similar spots on TLC. The two extracts were, therefore, mixed and subjected to column chromatography. This led to the isolation of a single constituent in major quantity. The compound was identified as **100** on the basis of spectroscopic studies.



100

The investigation of *Zanthoxylum simularis* was taken up because other species of *Zanthoxylum* had yielded novel alkaloids and coumarins. The plant was defatted and extracted with chloroform and ethanol. The ethanol extracts of the plants did not respond to the simple column chromatographic technique because of very close Rf values of constituents. The chloroform extract when subjected to exhaustive column chromatography gave two compounds, a limonoid and a

flavonoid. The structure of these compounds, identified as **101** and **106**, was established on the basis of spectroscopic studies and discussed at length in the thesis.



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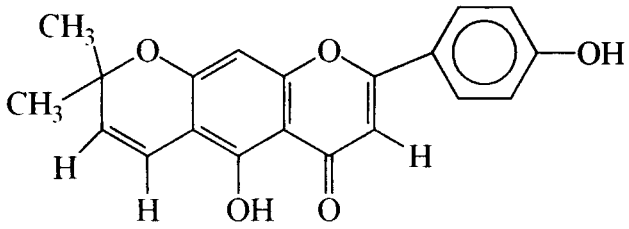
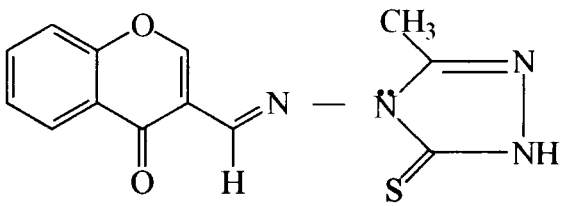


106

The major part of the thesis is devoted to synthesis of heterocycles from cheap and easily available starting materials such as 3-formylchromone, 2-amino-3-formylchromone and 2-ureidomethylene cyclohexane-1,3-dione. The reaction of 3-formylchromone was carried out with 4-amino-3-alkyl-5-mercapto-1,2,4-triazoles. The reaction afforded iminomethylchromones **38, 39** and **40**.



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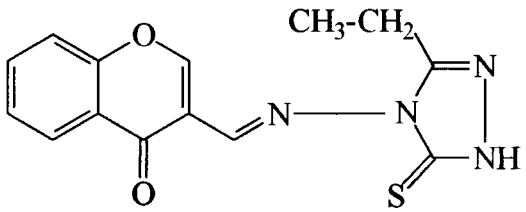

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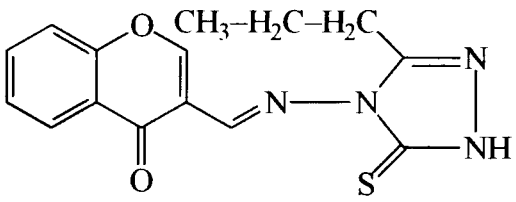
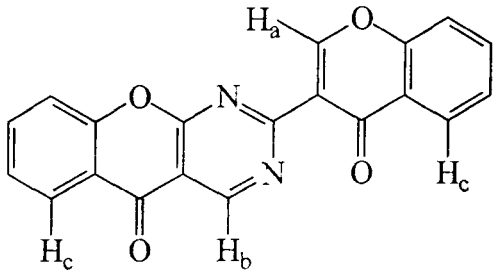


40

The structure of these compounds was inferred through spectral data. When exposed to nitrobenzene it gave rather unexpected products involving Diels Alder reaction. The spectroscopic studies suggest structures **42** and **43** for these products.



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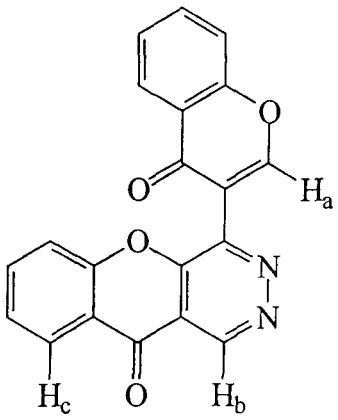
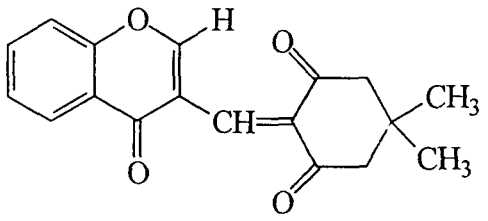


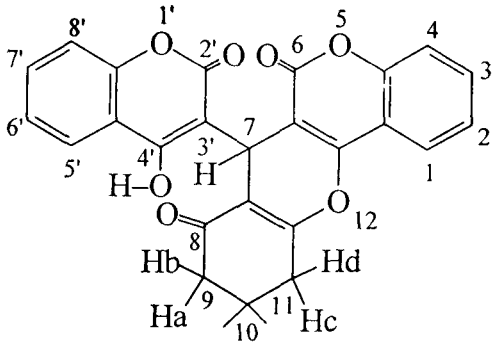
43

In another reaction 3-formylchromone was converted to **45** by carrying out the reaction with 5,5-dimethylcyclohexane-1,3-dione. The structure of the compound was determined through the application of spectral data.



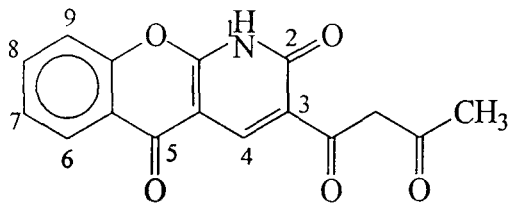
45

In another reaction 2-ureidomethylenehexane-1,3-dione was treated with 4-hydroxycoumarin to give **48.** The compound which has structure similar to the trimer obtained by the reaction of 4-hydroxycoumarin and triethylorthoformate in the synthesis of 3-formyl-4-hydroxycoumarin is novel and has shown biological properties.



48

2-Amino-3-formylchromone, synthesized under basic conditions from oxime of 3-formylchromone, was treated with compounds having nucleophilic properties. The compounds triacetic acid lactone, 5,5-dimethylcyclohexane-1,3-dione and 3-methyl-1-phenyl-5-pyrazolone were selected for this purpose. The reaction afforded compounds **62**, **68**, **78** and **81**. The structure of these compounds was determined on the basis of spectroscopic studies.



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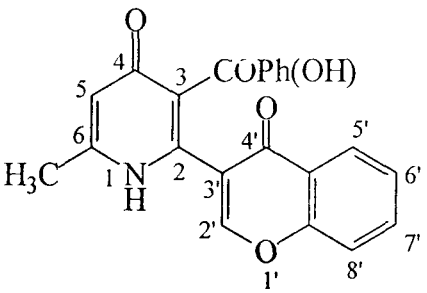

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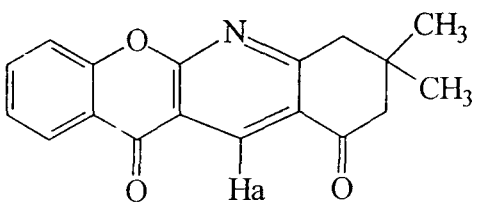

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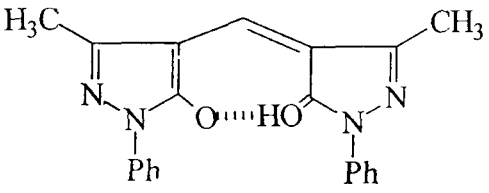

81

All the compounds except **101** and **106** were screened for antibacterial (Gram +ve and Gram –ve) and antifungal activities including *Candida albicans*. Some of them exhibited very good whereas others showed moderate activity.

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Certificate

This is to certify that the thesis entitled "Studies in Chemistry of O & N Heterocycles and Plant Products" submitted for the award of the Degree of Doctor of Philosophy (Ph.D.) in Chemistry to Aligarh Muslim University, Aligarh, is a record of bonafide research work carried out by **Ms. Gulrana Khuwaja** under my guidance. It is further certified that the thesis embodies the work of candidate herself and has not been submitted for any degree either of this or any other University. The present work is suitable for submission for the above mentioned purpose.

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CONTENTS

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Aligarh

June, 2006

Gulrana

(Gulrana Khuwaja)

PREFACE

The work described in the thesis is based in part on the synthesis of heterocyclic compounds from readily available starting materials and in part on the investigation of medicinally important plants. In the context of synthesis of heterocycles. different projects were under taken.

In the first. an attempt was made to obtain new heterocyclic compounds from cheap and easily available starting materials. The compounds selected were 3-formylchromone. 2-amino-3-formylchromone and 2-ureidomethylenecyclohexane-1,3-dione. 3-Formylchromone is readily converted to 2-(4-oxo-4H[1] benzopyran-3-yl) [1] benzopyrano (3,2-e) pyrimidin-5- (5H)-one) **42** and 1-(4-oxo-4H-[1] benzopyran-3-yl) [1] benzopyrano (3,2-d] pyridazin-5- (5H)-one **43** through 3(3-alkyl-5-mercapto-1,2,4-triazolyliminomethyl) chromones **38-40**. The structure of these heterocycles was inferred through spectroscopic data. 2-Amino-3-formylchromone is converted to polyketomethylene compounds by treatment with triacetic acid lactone under different reaction conditions. This led to the formation of 3-acetoacetyl-5-oxo-5H-1-benzopyrano-[2,3-e] pyridin-2-one **62** and 6-methyl-2-(4-oxo-4H-1-benzopyran-3-yl)-3-(2'-hydroxybenzoyl)-4-pyridone **68** the structure of which though complicated mechanistically were established through spectroscopic

studies. 2-Amino-3-formylchromone similarly is converted to 3,3-dimethyl-5-oxo-cyclohexa[2,3-b]-azaxanthone **78** and methylidene-bis-4, 4 -(3-methyl-5-oxo-1-phenylpyrazole **81** with 3-methyl-1-phenyl-5-pyrazolone and 5,5-dimethylcyclohexane-1,3-dione. These compounds were synthesized under mild conditions with improved yield and showed biological activities. In another reaction ureidomethylene cyclohexane-1, 3-dione was treated with 4-hydroxycoumarin. The reaction was carried out in an effort to synthesize coumarin **47** through Knoevenagel condensation type of reaction. The results, however, showed formation of 7-(4-hydroxycoumarin-3-yl)-10, 10-dimethyl-8-oxo-8, 9,10,11-tetrahydropyrano [3,2-c] coumarin **48**. The structure was established through spectroscopic data.

In the second part of the thesis efforts were directed towards the separation of active principles from *Piper cubeba, Piper chaba and Zanthoxylum simularis*. The acetone extract of *Piper chaba* yielded a compound which appeared on TLC plate fluorescent. The compound, melting point 240°C showed a strong band at 1660 cm^{-1} in its IR spectrum. This indicated the possibility of a flavonoid (positive Shinoda test). Its structure, however, could not be established because of insufficient amount of the compound which was needed for getting more exhaustive spectral data. The petrol and benzene extracts of *Piper cubeba* were subjected to column chromatography and yielded cubebin

100 as the only active principle in major quantity. The investigation of *Zanthoxylum simularis* was taken up because other species of this plant had yielded novel alkaloids and coumarins. In the present investigation efforts were made towards separation of a number of phenolic compounds which on TLC plates of the extracts appeared as fluorescent spots. Chromatographic work up of the chloroform extract of *Zanthoxylum simularis*, afforded limonin **101** and flavone **106**. This is the first report on isolation of these two compounds from *Z. simularis*. The third chapter is devoted to the antimicrobial screening of the compounds synthesized in the laboratory and isolated from the plant. All compounds showed very good antimicrobial activity against bacteria and fungi including *Candida albicans*.

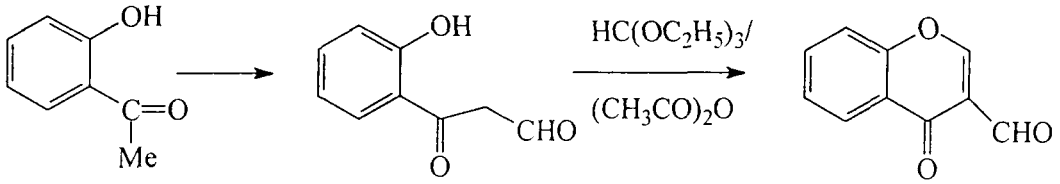
CHAPTER 1

Synthesis and Characterization of Heterocycles

1. Introduction

Chromone and its derivatives have been reported to perform important biological activities such as oestrogenic, antibacterial, anti-inflammatory, anti-spasmolytic, antitumor and anti-hepatotoxic activities[1-5]. Thus, it has always attracted the attention of chemists for synthesis of new heterocycles from it.

A number of methods have been reported in the literature for the preparation of 3-formylchromone. Eiden et al have synthesized it for the first time by formylating 2-formyl-2'-hydroxyacetophenone derived from o-hydroxyacetophenone using ethyl orthoformate and acetic anhydride[6].

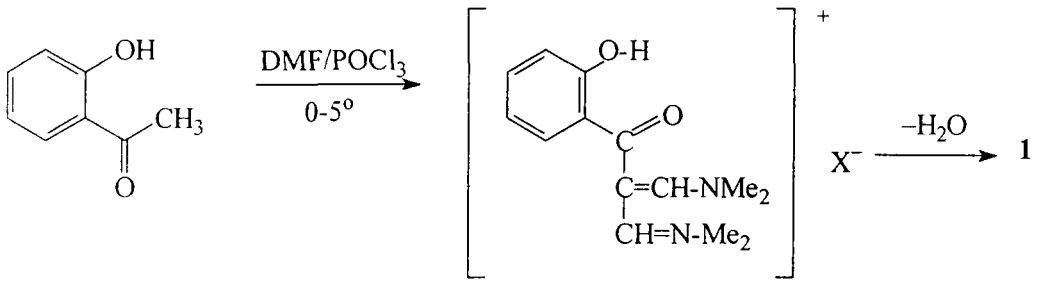


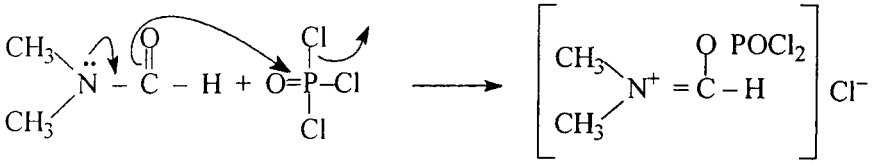
1

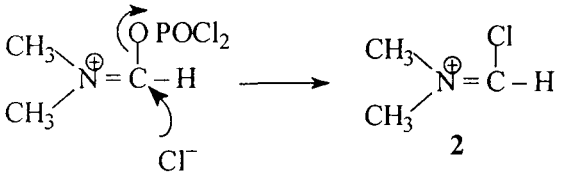
This method however is not good enough as it affords **1** with poor yield. The compound **1** also has been synthesized from 2, 2-difluro-4-methylnaphtho-1,3,2-dioxaborin by the Vilsmeier Haack reaction[7].

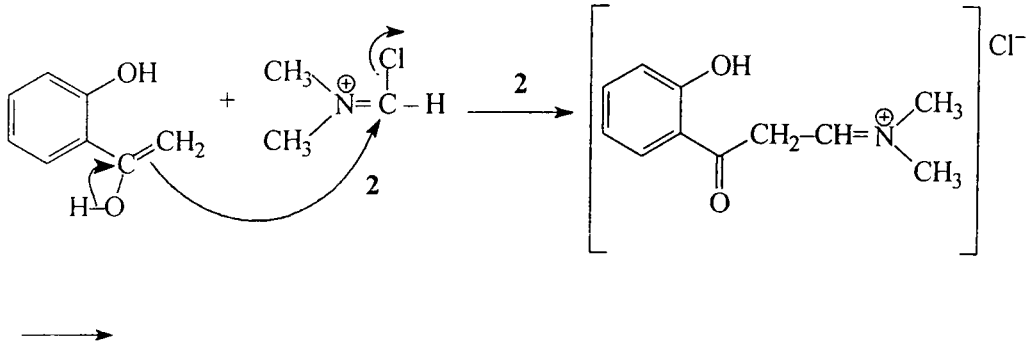
Though various methods are known for carrying out C-formylation Vilsmeier Haack reaction seems best as this reagent is very mild and can be applied to even polyfunctional molecules. In addition to this, Vilsmeier Haack reaction affords **1** in good yield and less time

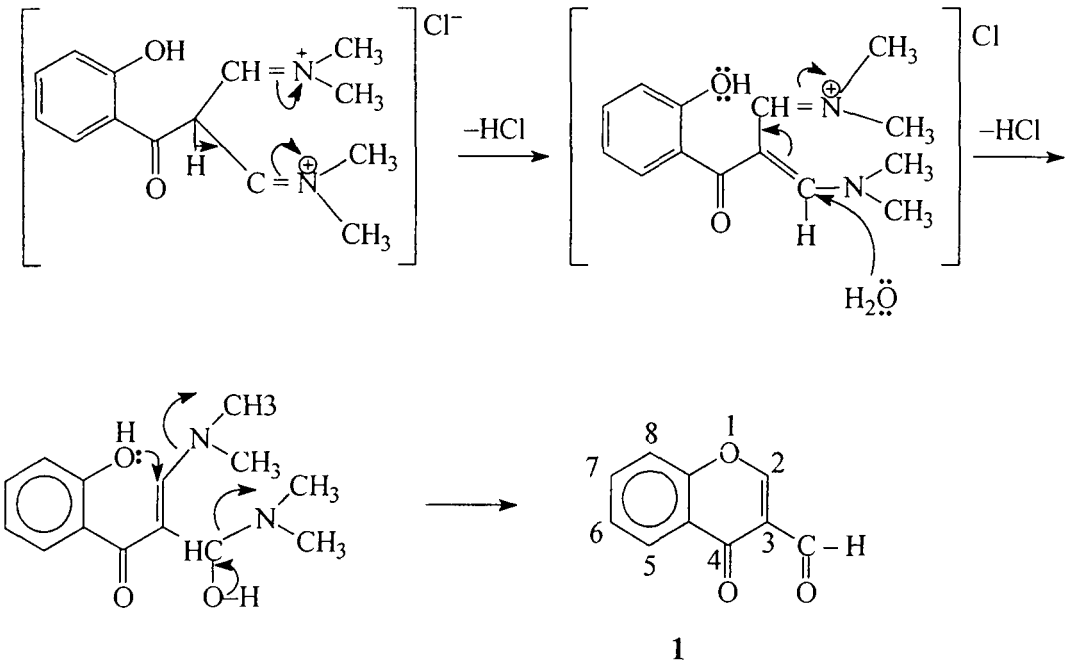
period. Thus, Nohara et al[8] reported for the first time synthesis of **1** by Vilsmeier Haack reaction of o-hydroxyacetophenone using DMF and $POCl_3$ (Scheme 1).









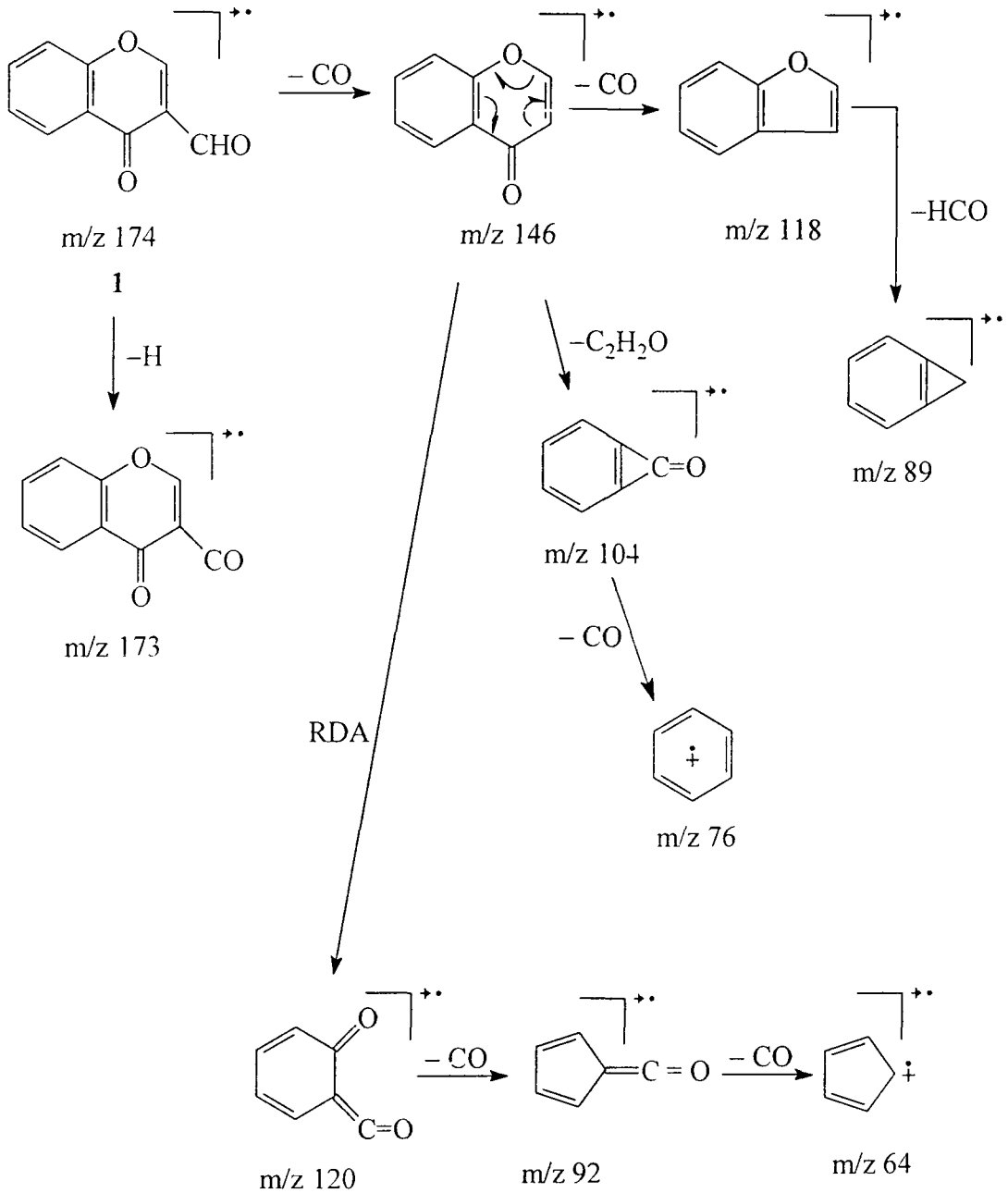


Scheme-1

Recently Linghua and co-workers[9] studied several molar ratios of substituted o-hydroxyaccetophenone-$POCl_3$-DMF such as 1:1:1, 1:3:3, 1:3:6 and 1:6:12 for synthesis of 6-alkyl and halo substituted 3-formylchromone. They found that in the ratio 1:6:12, the yield of the product was best. even higher than that reported earlier. Some authors have also reported synthesis of hydroxy substituted 3-formylchromone in connection of synthesis of 2-oxopyranochromone-3-carboxaldehyde, by choosing appropriately hydroxy substituted o-hydroxy acetophenone and DMF/$POCl_3$[10].

In the IR spectrum of **1** a band at 1692-1700 cm^{-1} is due to aldehydic group. The chromone carbonyl group usually appears as a strong band at 1645-1665 cm^{-1}.

The ^{1}H NMR spectrum of **1** gives singlets at δ 10-11, 8.4-8.5 for aldehydic and C-2 protons respectively. The double doublet of C-5 proton is usually found at δ 8.2-8.3. The remaining three aromatic protons are in the form of multiplet in the aromatic region δ 7.4-7.8. In the mass spectrum the molecular ion peak at m/z 174 loses CO to give the base peak at m/z 146 which again looses CO to give the peak at m/z 118. Another mode of breaking down of the m/z 146 ion occurs through loss of C_2H_2O and CO groupings to give peaks at m/z 76. The ion at m/z 146 further suffers RDA cleavage to give fragment ions at m/z 120 which eliminates CO to form fragment ions at m/z 92 and 64 (Scheme-2). The m/z 146 ion produced from **1** seems to be same as the molecular ion of chromones[8] but the degradations giving peaks at m/e 104 and m/z 76 are not important pathways in the latter. This mode of beak down which differs from those of flavones and chromones seems to be chracteristic feature of **1**.


- CO
- CO
-HCO
m/z 174
1
m/z 146
m/z 118
-H
$-C_2H_2O$
m/z 89
m/z 173
m/z 104
- CO
RDA
m/z 76
- CO
- CO
m/z 120
m/z 92
m/z 64

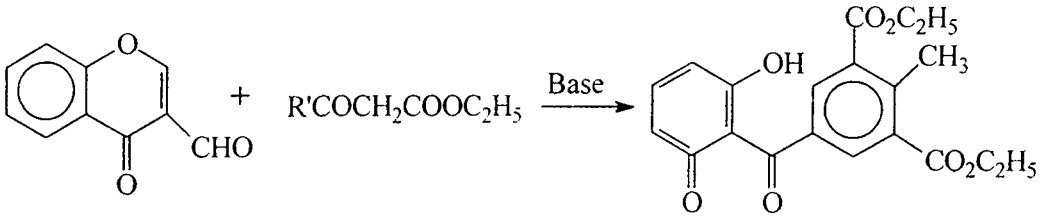
Scheme-2

2. Theoretical

3-Formylchromones are useful synthons for the synthesis of a large number of heterocycles as it contains several electron deficient centres namely C-2, C-4 and carbon of formyl group which can be modified into various types of derivatives by the attack of nucleophile. In the context of further work on 3-formylchromone, a survey of literature of about 30 years was done and some relevant examples are discussed below.

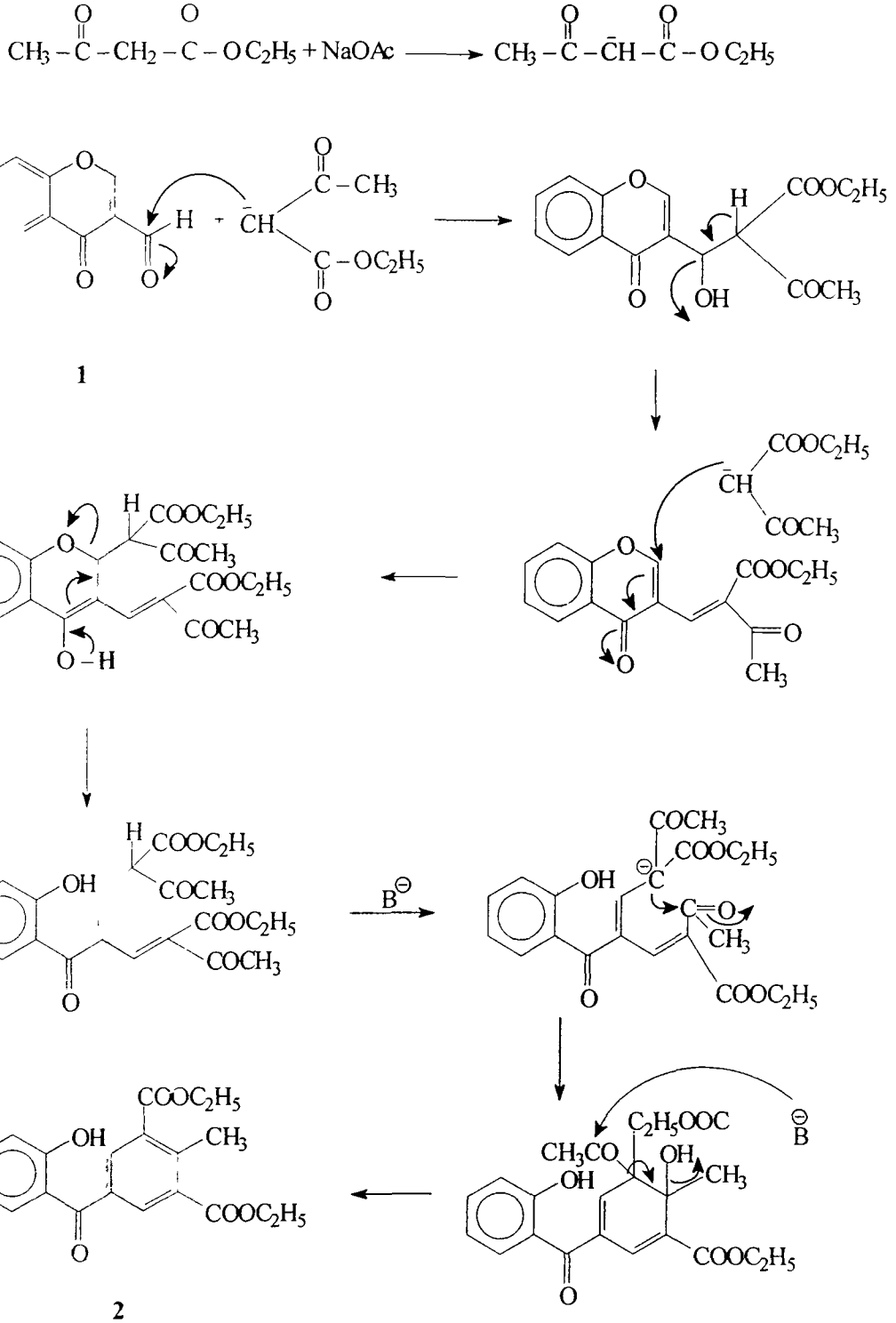
2.1 Reaction of 3-formylchromone (1) with active methylene compounds:

One of the earliest example in this category is synthesis of o-hydroxyacetophenone **2** from 3-formylchromone **1** and ethylacetoacetate in the presence of a base [11].



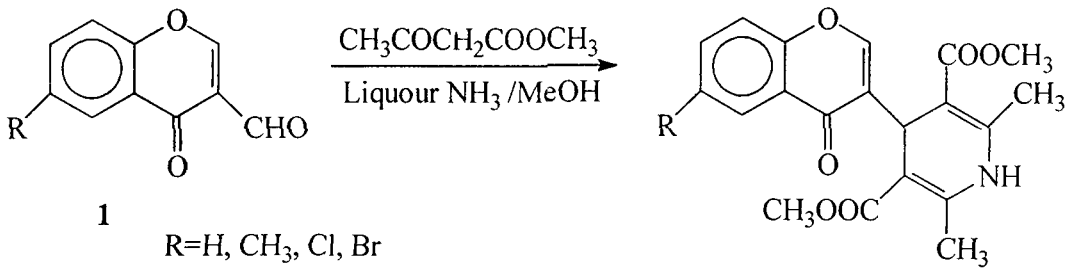
1 **2**

The mechanism involves initial condensation of **1** with ethyl acetoacetate followed by Michael addition, opening of pyrone ring and subsequent cyclization (Scheme-3).



Scheme-3

An extension to such type of reaction is the treatment of **1** with methyl acetoacetate in presence of ammonia to give dihydropyridine derivatives **3**[12].

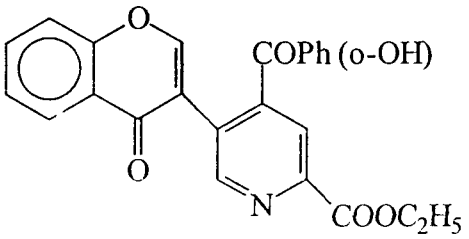


1

R=H, CH_3, Cl, Br

3

Another interesting example in this category is the condensation of **1** with amino esters such as glycine ester in the presence of a catalytic amount of p-toluenesulfonic acid. The reaction mixture gave a mixture of pyridine **4** and pyrrole **5**[13].



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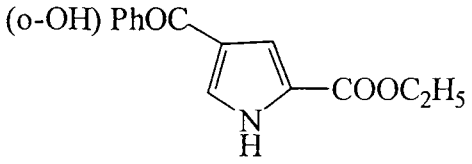
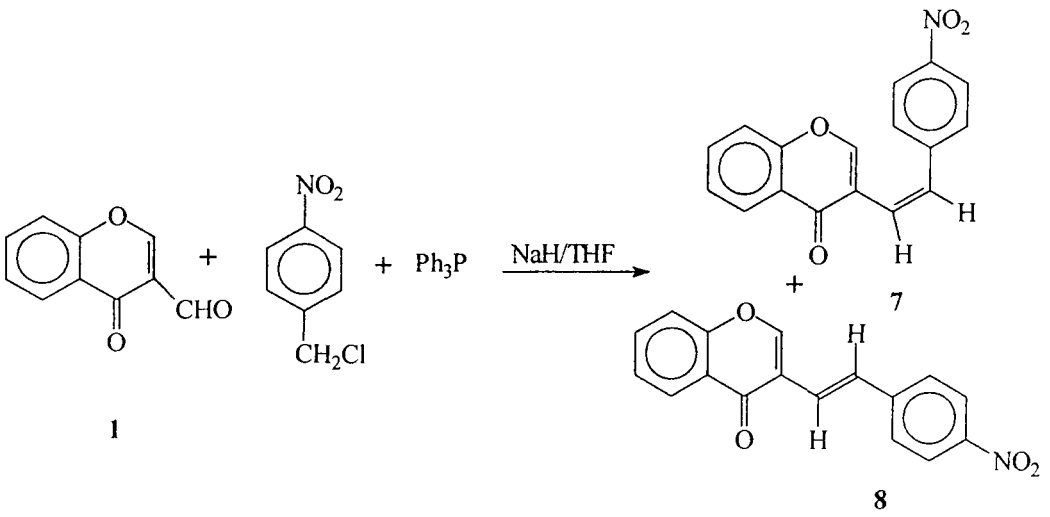


5

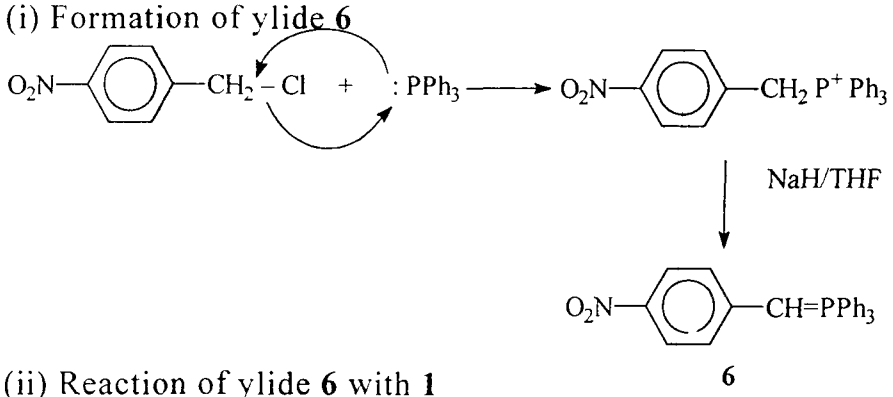
2.2 Synthesis of Styryl chromones:

Styrylchromones belong to a group of oxygen heterocycles and are reported to exhibit biological properties[14]. Heck reaction[15] used for the synthesis of styrylchromone from 3-bromochromone with styrene in the presence of Pd $(OAc)_2$, tri-o- tolylphosphine and $(Et)_3N$ in DMF has some disadvantages as the method has been used for the formation of only unsubstituted 3-styryl chromones and taking polyhalogenated chromone derivatives, the coupling of several alkenes to the chromone systems at the halogenated sites takes place to give undesired product. In this connection Portugees authors have reported synthesis of 3- styryl chromones from **1** and benzylic ylides (Witting reaction)[16]. The reaction involves treatment of benzylidene triphenyl phosphorane **6** with **1** to give a mixture of *cis* and *trans* 3- styryl chromones, **7** and **8** (Scheme 4).



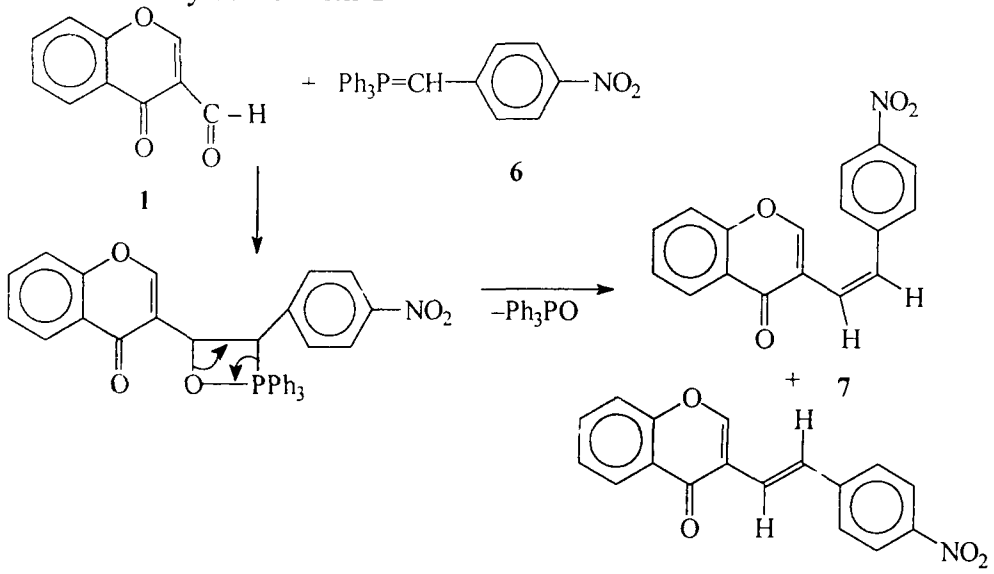
The reaction mechanism involves following steps:

(i) Formation of ylide **6**



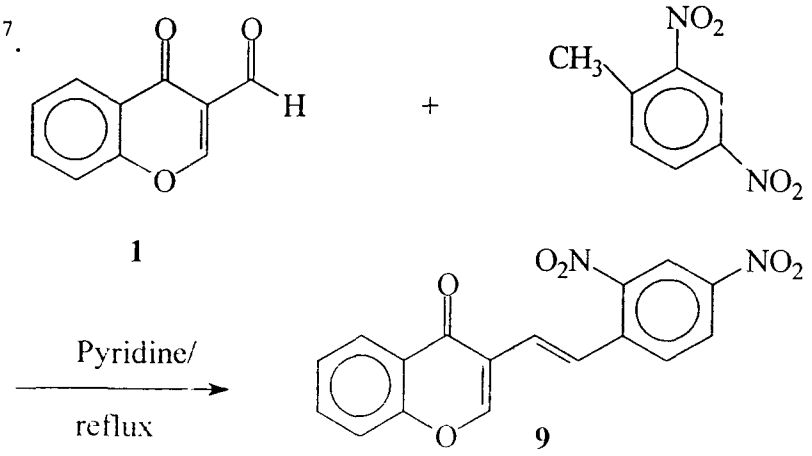
6

(ii) Reaction of ylide **6** with **1**



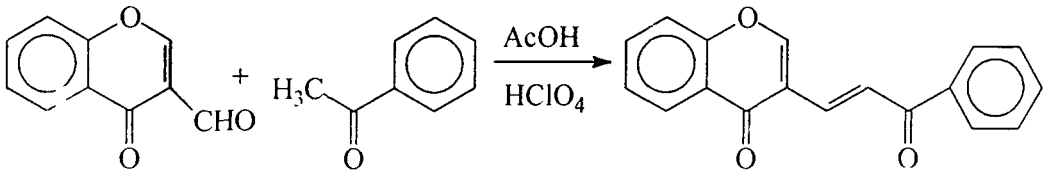
Scheme-4 **8**

Another simple transformation of **1** to styrylchromone **9** involves treatment of **1** with 2,4, dinitro toluene in the presence of base such as pyridine[17].



2.3 Synthesis of chalcones:

The chalcones are important precursors of flavonoids and isoflavonoids[18]. A large number of chalcones and their derivatives have been prepared from Claisen-Schmidt condensation of aldehydes with methyl ketones under basic conditions[19]. These compounds have shown *in vitro* anti-malarial activity against chloroquine sensitive and chloroquine resistant strains of *Plasmodium falsciparum*[20]. A variety of chalcone derivatives also have been reported for acting as potent tyrosinase inhibitors, antioxidants and are thus used as new depigmentation agents[21]. Recently Thskaev et al[22] have reported conversion of **1** to cholcone **10** with acetophenones under acidic conditions using acetic acid and perchloric acid and have shown a variety of biological activities.

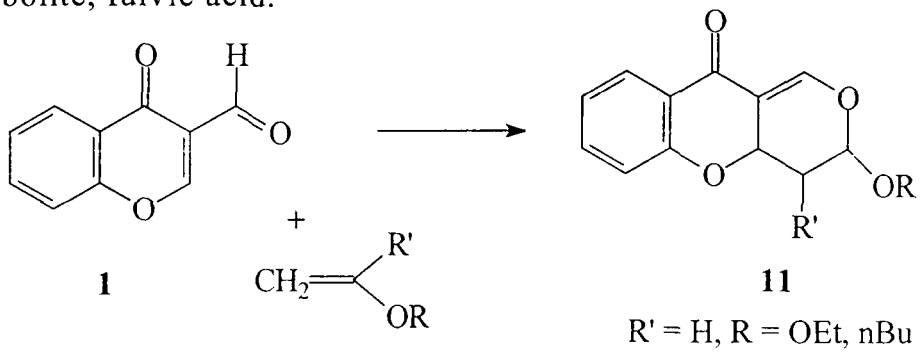


1 **10**

2.4 (4π+ 2π) Cyclo addition reactions of 1

Due to presence of carbonyl group at position **3**, which may conjugate with double bond, **1** also functions as diene in (4π+2π) cycloaddition reaction with dienophiles. The intermediate adduct as obtained can be transformed to other useful heterocycles either by rearrangement[23] or through ring opening reactions[24]. An interesting

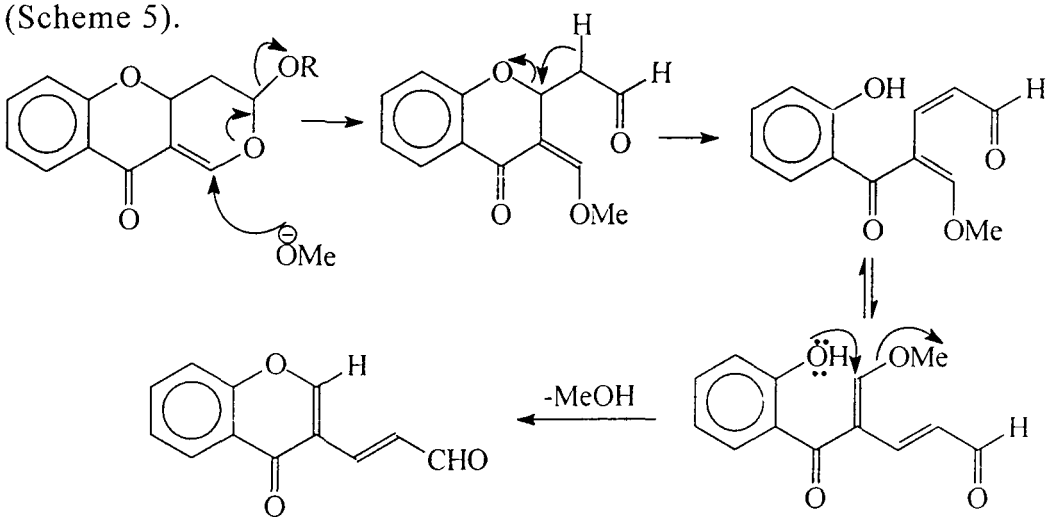
example in this context is the reaction of **1** with vinyl ethers to give **11** which has the heterocyclic unit similar to that present in fungal metabolite, fulvic acid.[25]



1 **11**

R' = H, R = OEt, nBu
R' = Me.

The adduct **11** has been converted to aldehyde **12** with methoxide ion through ring opening followed by rearrangement and cyclization[26] (Scheme 5).

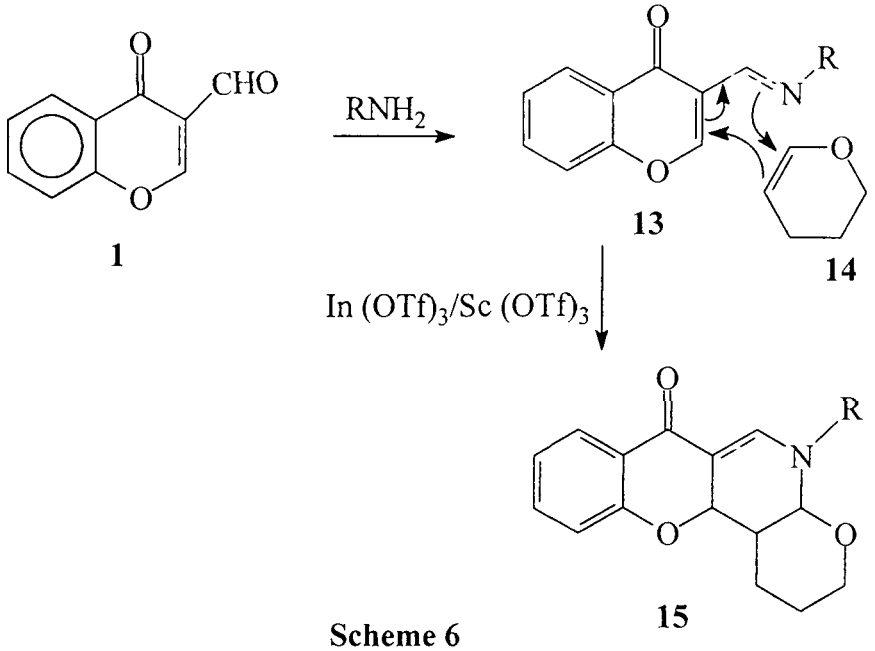


12

Scheme 5

The aldehyde **12** has also been synthesized by Ghosh et al[27] indirectly from 3-substituted chromone.

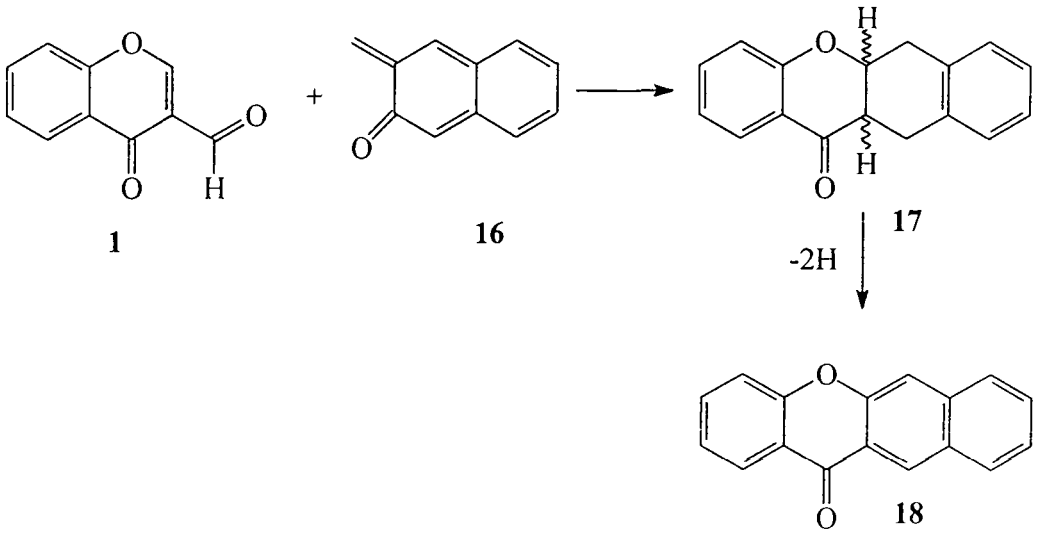
Sandhu et al[28] synthesized Shiff's base **13** of **1** and then used it as a diene in the reaction with dienophile **14** to form the adduct **15** in the presence of triflate as a catalyst (Scheme 6).



Scheme 6

The compound was identified on the basis of ^{1}H NMR which showed disappearance of diagnostic peak of H-2 proton at δ 8.65 in **1** and presence of upfield signal at δ 5.34. This confirmed that cycloaddition had occurred at C-2 position of the chromone unit.

Diels Alder reaction of 3-formylchromone with o-benzoquinone dimethane **16** forms bezoxanthones **18** through oxidation of cycloadduct **17**.

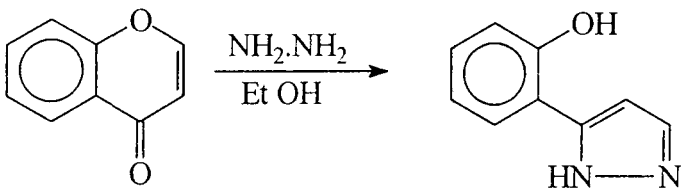


Scheme 7

Xanthones[29], which are naturally occurring compounds and are present as minor constituents in plants, can be synthesized, thus, in one step reaction as shown in Scheme 7.

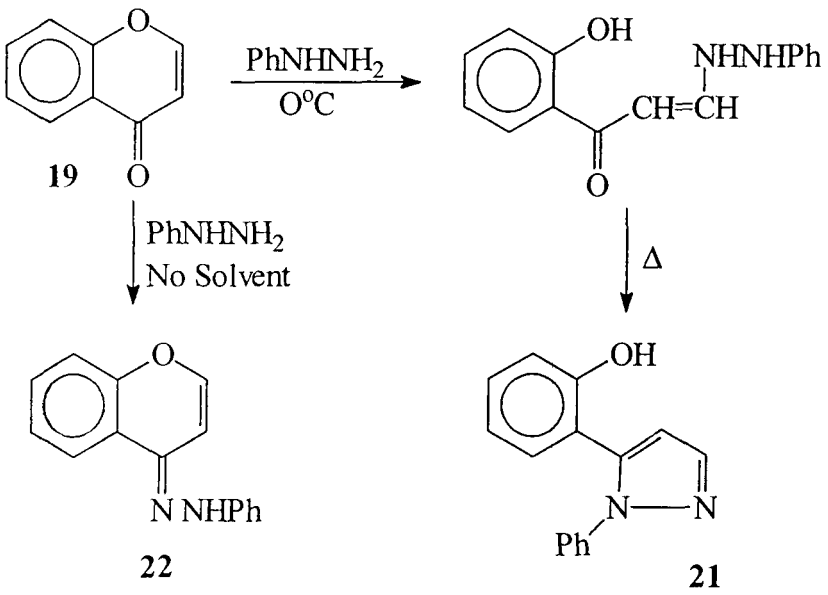
2.5 Ring Opening Reactions

Chromone and its derivatives react with nitrogen bases and form the products by opening of pyrone ring followed by cyclisation. Schonberg et al[30] refluxed a mixture of chromone **19** and hydrazine hydrate in ethanol and believed that the product was hydrazone (absence of chromone carbonyl group in IR spectrum) but Baker et al[31] showed that the product was pyrazole **20** (absence of diagnostic signal of H-2 proton of chromone moiety).



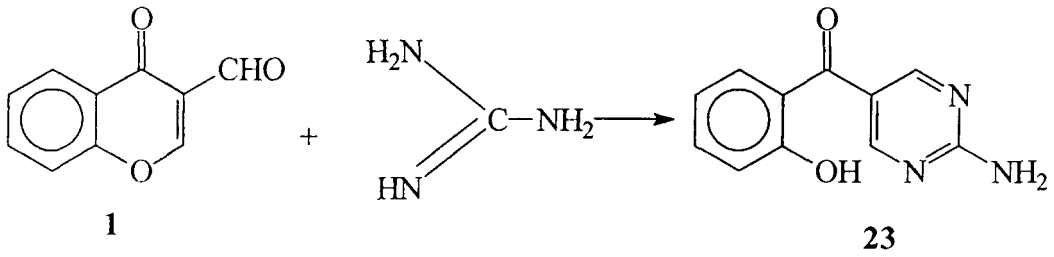
19 **20**

Phenylhydrazine also causes ring cleavage of chromones but under some conditions the phenylhydrazone **22** is also formed[32,33] Scheme 8



Scheme 8

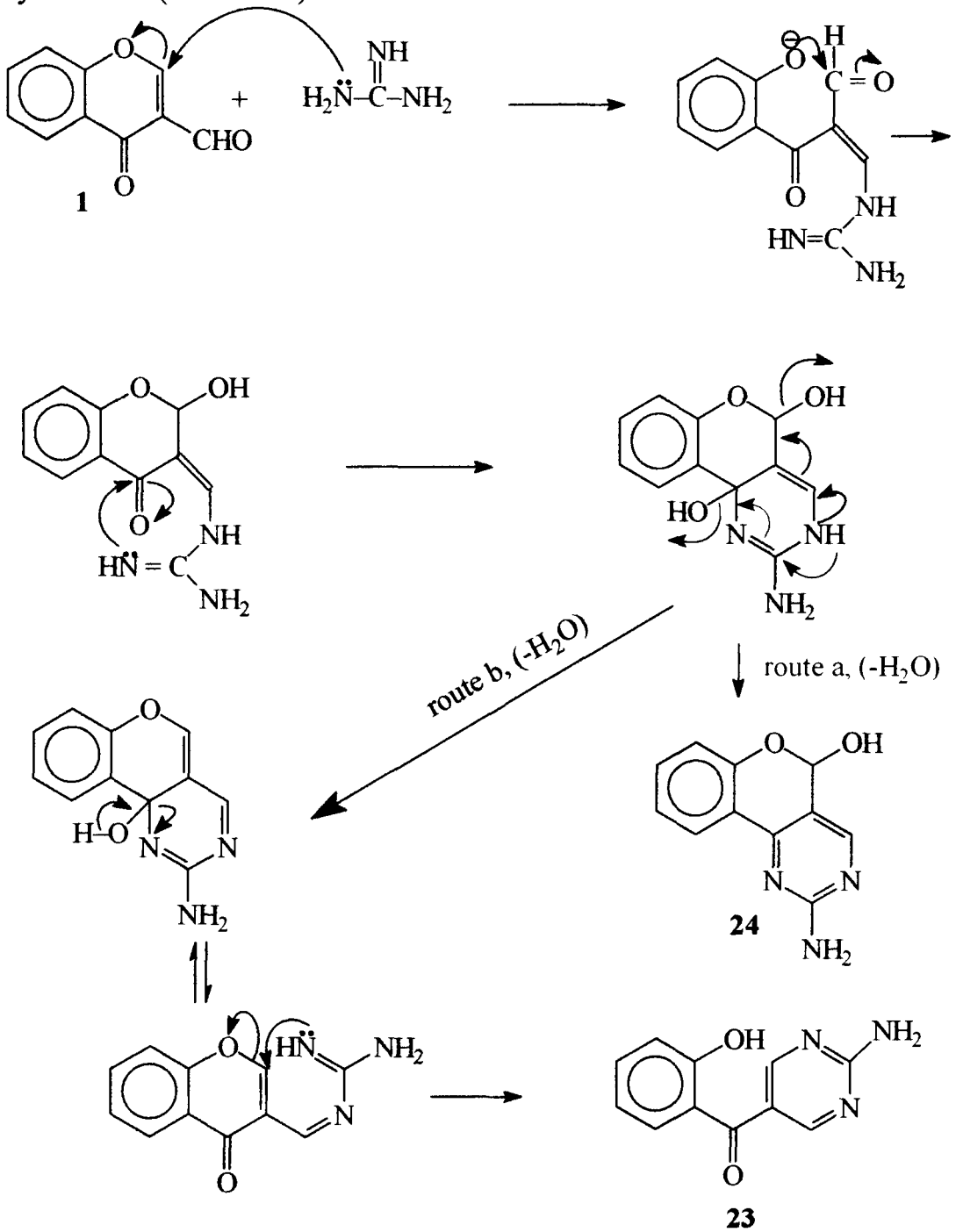
Treatment of **1** with guanidine in the presence of ethoxide affords pyrimidine **23**[34].



The authors explained the formation of **23** by 1,2 addition of guanidine, generated from its salt by alkali or alkoxide, to the aldehydic group of **1** followed by ring opening and subsequent cyclization. This, however, effects the yield of the product as extensive degradation takes place in the presence of alkali which also changes the course of the reaction.

Ghosh et al[35] obtained **23** along with **24** by the reaction of **1** with guanidine without using alkali or alkoxide. This method afforded pyrimidine derivatives in good yield.

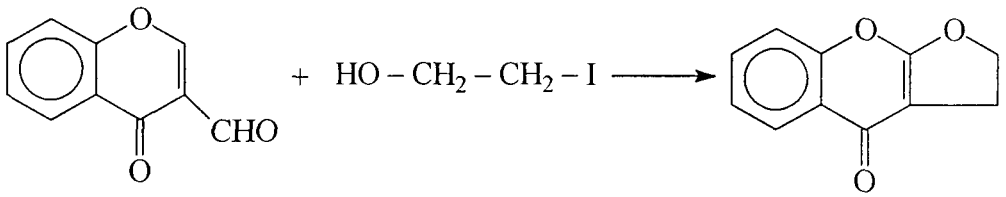
The formation of **23** and **24** take place by initial 1,4 addition of nucleophile to pyrone ring followed by subsequent elimination and cyclization (Scheme 9)



Scheme 9

2.6 Synthesis of tetrahydrofuran derivative

In another useful transformation **1** was treated with halohydrin to give tetrahydro furan derivative**25**[36]. This reaction was investigated when an effort was made to carry out hydroxyethylation of **1** under basic conditions.

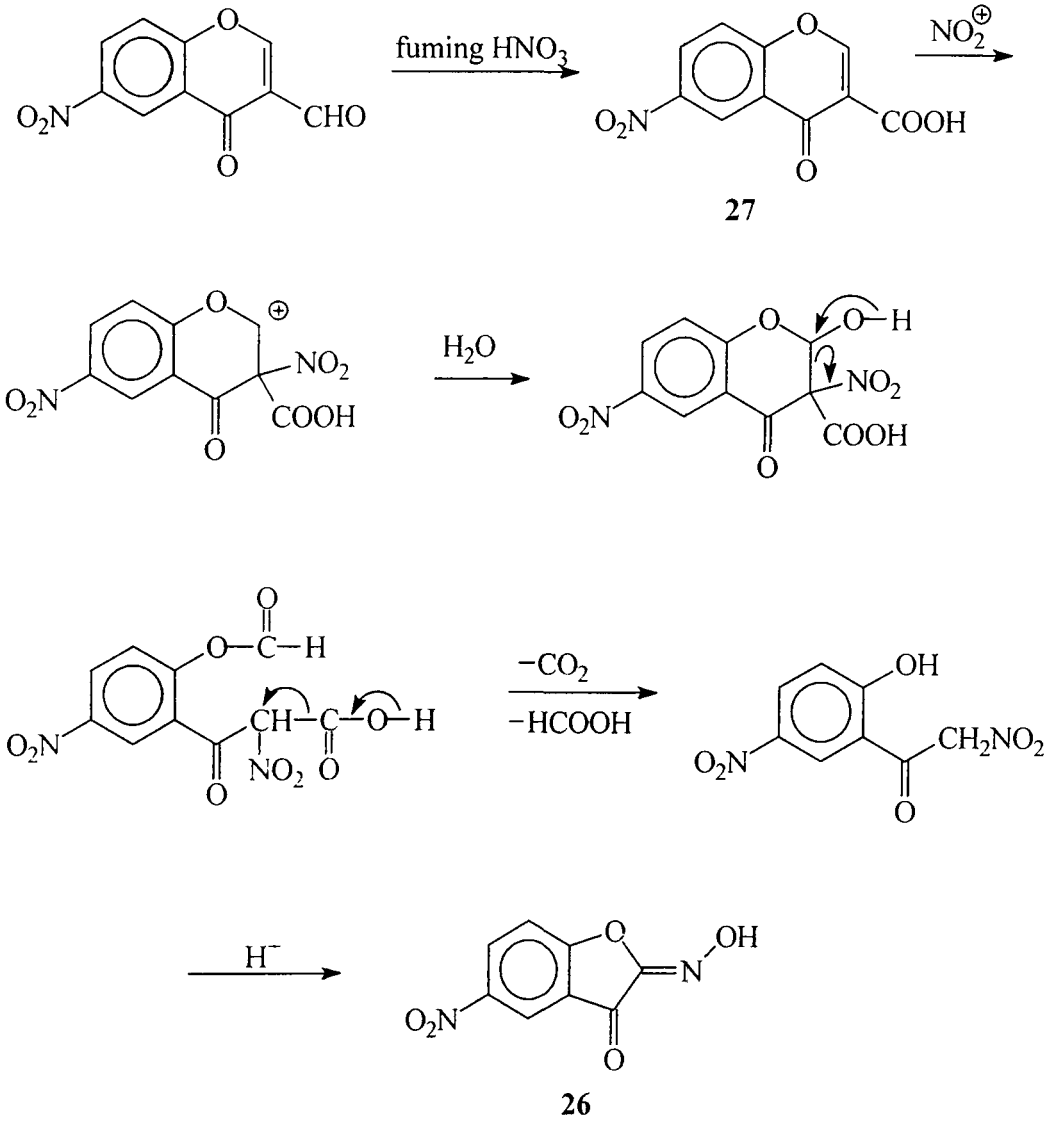


1 **25**

The reaction involves attack of halohydrin on C-2 carbon followed by cyclization and deformylation.

2.7 Synthesis of 5-nitro-2,3-benzofurandione-(z)-2-oxime

In an attempt of synthesize 6-nitro-3-formylchromone[8] using fuming nitric acid, Curran et al[38] reported formation of oxime **26** as shown in Scheme 10. The mechanism involves oxidation of 3-formylchromone to carboxylic acid[37] **27** which undergoes electrophilic addition by nitronium ion followed by retroaldol reaction, hydrolysis and decarboxylation.

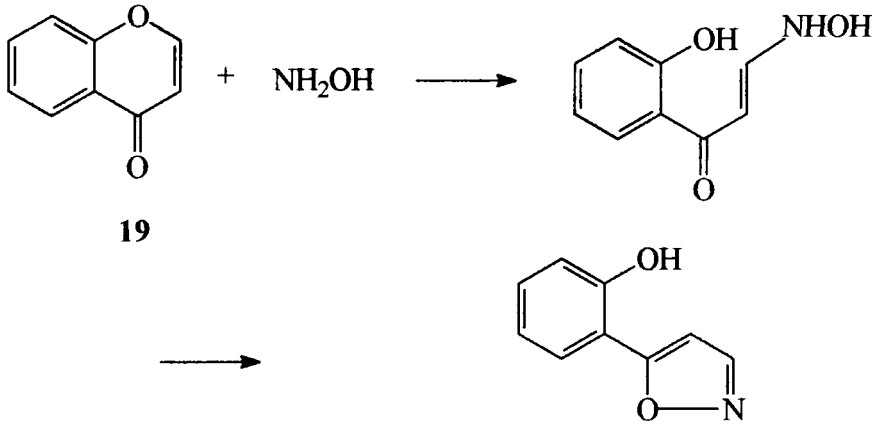

fuming HNO3
NO2⊕
CHO
COOH
27
H2O
NO2
COOH
−CO2
−HCOOH
OH
CH2NO2
H−
OH
26

Scheme 10

3. Discussion

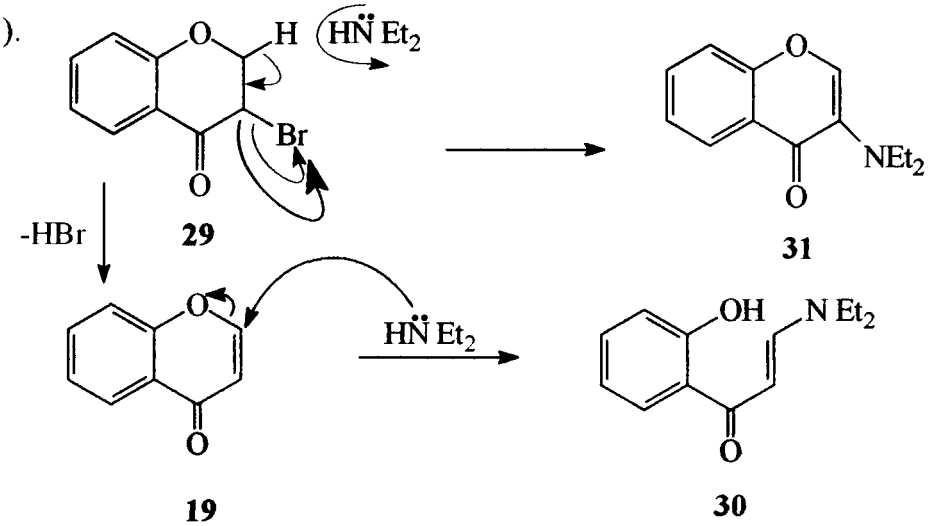
3.1 The reaction of 3-formylchromone with 3-alkyl-4- amino – 5-mercapto –1,2,4-triazole.

Chromones usually undergo opening of pyrone ring via nucleophilic attack at C-2. Thus, chromone **19** reacts with nitrogen nucleophiles such as hydroxyl amine to give **28**.



19 **28**

Aniline[39] also behaves in a similar manner. When diethylamine is allowed to react with 3-bromochromanone, **29** the product is **30** and not **31**. The formation of **30** involves chromone, **19** as intermediate which undergoes ring opening via nucleophilic attack of diethylamine (Scheme 11).

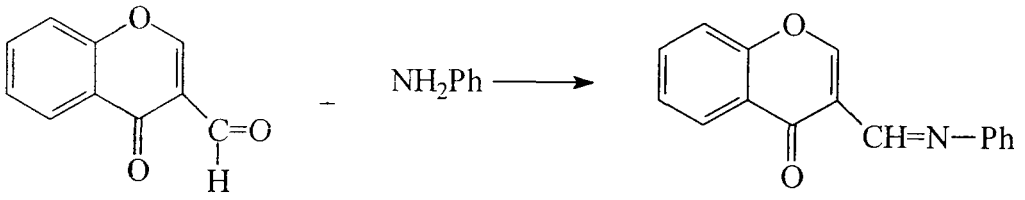


29 **31**

19 **30**

Scheme 11

The reactivity of chromone system towards nucleophiles is however, changed when certain substituent are present at position 3. Thus, the reaction of 1 with aniline proceeds without opening of the chromone ring to give 32[40].

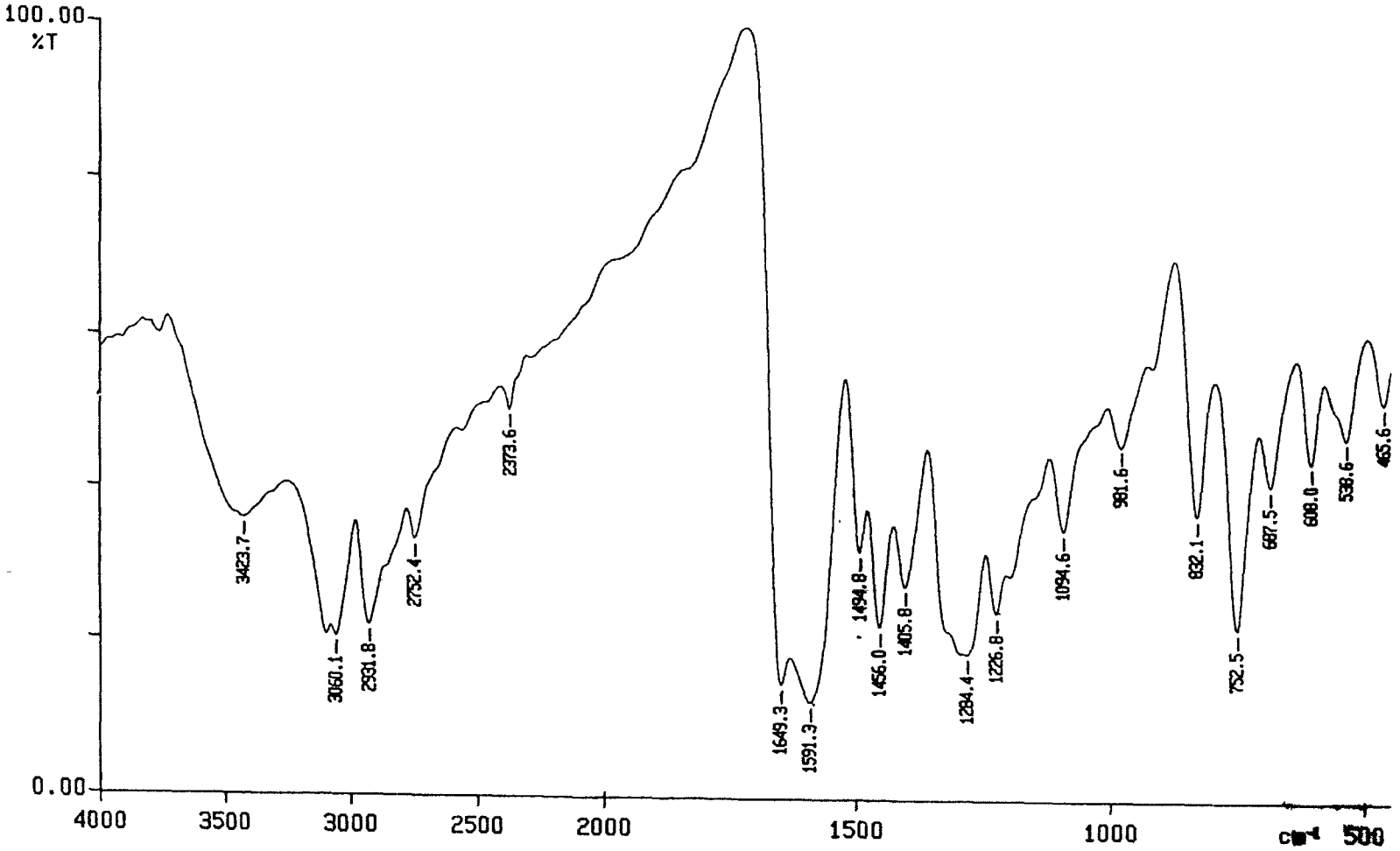


1 32

It appeared, therefore, interesting against this background to study the reaction of **1** with alkyl substituted 4-amino-5-mercapto- 1,2,4-triazole. Thus, the reaction with different types of triazoles viz. 4-amino-3-methyl-5-mercapto-1,2,4-triazole **33,** 4-amino-3-ethyl-5-mercapto- 1,2,4-triazole **34**, and 4-amino-3-propyl-5-mercapto-1,2,4-trizole **35**, afforded T3Fc(B), 3FcTP, 3FcTB and their structures are discussed below.

3.1.1 The reaction of 1 with 4-amino-3-methyl-5-mercapto-1,2-4-triazole:

The compound shows M^++1 peak at m/z 287 in its mass spectrum (Fig. 1). The IR spectrum (Fig. 2) exhibits a broad band at 3424 cm^{-1} which is assigned to NH group. The IR spectrum also displays a strong



02/07/22 11:11 smg
X: 4 scans, 4.0cm-1, flat, smooth, abex

Fig. 2

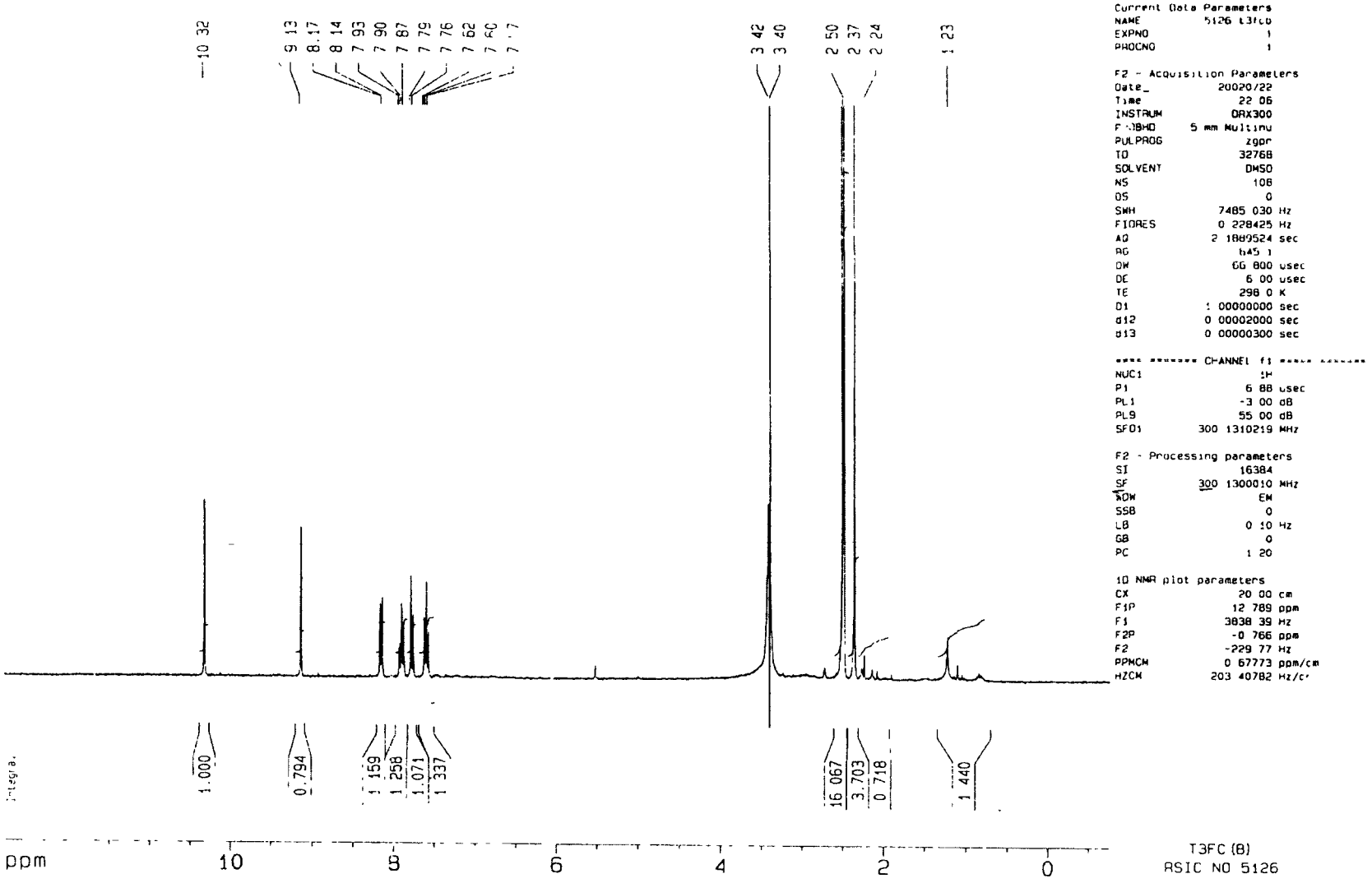
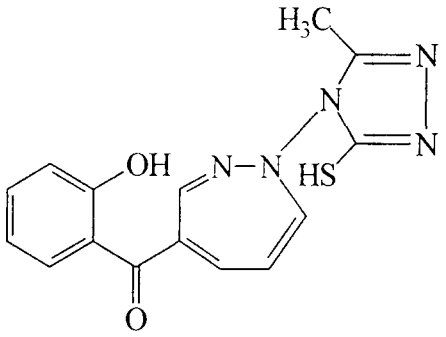

10 32
9 13
8.17
8 14
7 93
7 90
7 87
7 79
7 76
7 62
7 60
7 7
3 42
3 40
2 50
2 37
2 24
1 23
1.000
0.794
1 159
1 258
1.071
1 337
16 067
3.703
0 718
1 440
ppm
10
8
6
4
2
0
Current Data Parameters
NAME 5126 t3fcb
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20020/22
Time 22 06
INSTRUM DRX300
PULPROG zgpr
TD 32768
SOLVENT DMSO
NS 108
DS 0
SWH 7485 030 Hz
FIDRES 0 228425 Hz
AQ 2 1889524 sec
RG b45 1
DW 66 800 usec
DE 6 00 usec
TE 298 0 K
D1 1 00000000 sec
d12 0 00002000 sec
d13 0 00000300 sec
CHANNEL f1
NUC1 1H
P1 6 88 usec
PL1 -3 00 dB
PL9 55 00 dB
SFO1 300 1310219 MHz
F2 - Processing parameters
SI 16384
SF 300 1300010 MHz
WDW EM
SSB 0
LB 0 10 Hz
GB 0
PC 1 20
1D NMR plot parameters
CX 20 00 cm
F1P 12 789 ppm
F1 3838 39 Hz
F2P -0 766 ppm
F2 -229 77 Hz
PPMCM 0 67773 ppm/cm
HZCM 203 40782 Hz/cm
T3FC (B)
RSIC NO 5126

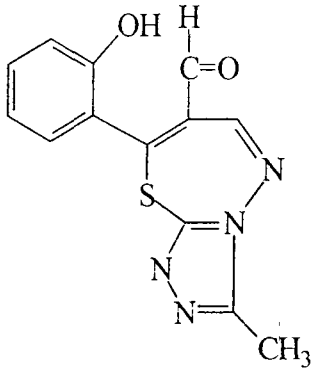
Fig. 3

absorption band at 1649 cm^{-1} and a moderate band at 1284 cm^{-1}. These bands are due to chromone carbonyl and C=S functional groups. Since the compound does not give the characteristic colour test with $FeCl_3$, the opening of the chromone ring is ruled out. Therefore, a structure like **36** cannot be assigned to the compound.



36

The other structure **37** compatible with the value of M^+ is again not possible because of absence of aldehydic band at 1700cm^{-1} in the IR spectrum and negative test with ferric chloride.

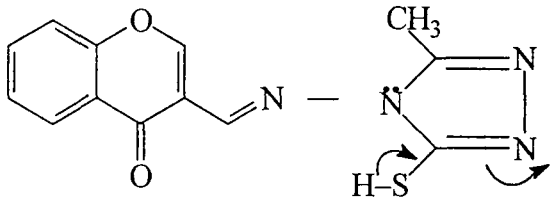


37

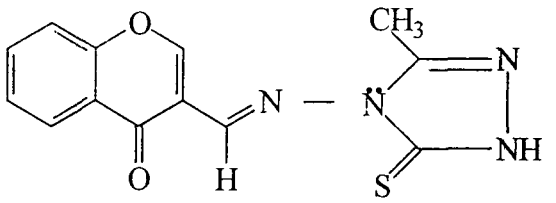
The nmr spectrum (Fig. 3) of compound shows presence of methyl group as a sharp singlet at δ 2.37. The doublet of H-5 proton of

chromone nucleus is clearly seen at δ 8.17. The characteristic singlet of H-2 proton of chromone nucleus at δ 9.13 is at rather high value and this may be due to presence of electron withdrawing triazole nucleus at C-3. The most ambiguous feature of the nmr spectrum is down field singlet at δ 10.37 for imine proton, as in literature the resonance of this proton usually appears at δ7.5 to 8.7.[41]

On the basis of these spectral features one arrives at structure **38** for compound which prefers to be present in the form **38a** involving thiol-thione tautomerism.

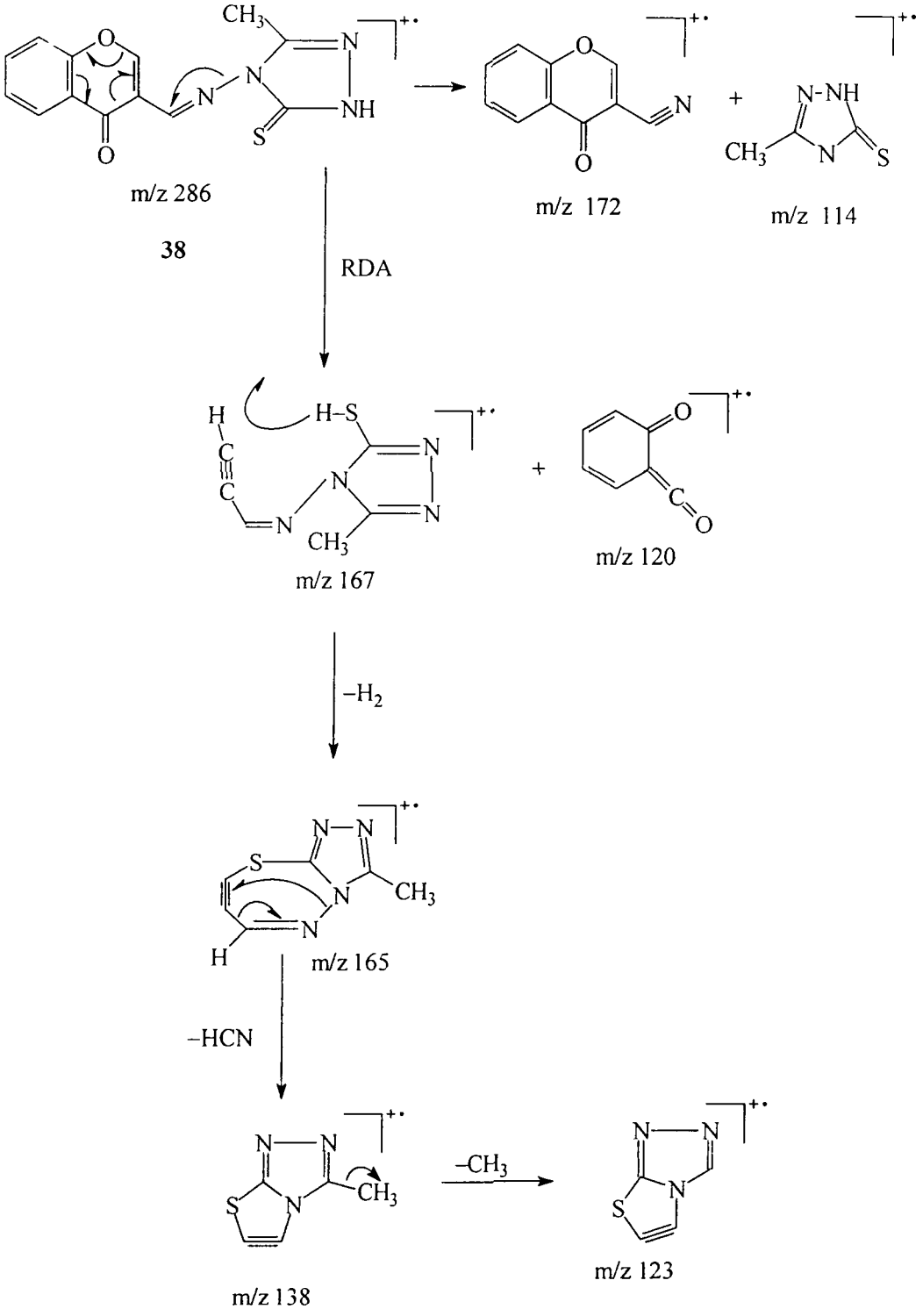


38



38a

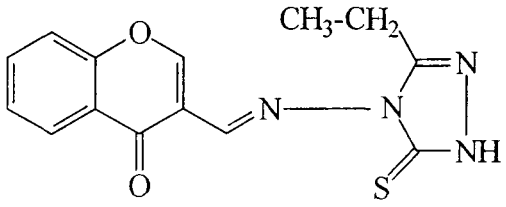
The mass spectrum (Fig. 1) is also consistent with structure **38** showing fragment ions at m/z 107, 120, 172, 165 as shown in Scheme 12


m/z 286
38
RDA
m/z 172
m/z 114
m/z 120
m/z 167
$-H_2$
m/z 165
-HCN
$-CH_3$
m/z 138
m/z 123

Scheme 12

3.1.2 The reaction of 1 with 4-amino-3-ethyl-5-mercapto-1,2,4-triazole:

The compound **39** shows (M^++1) at m/z 301in its mass spectrum (Fig. 4).

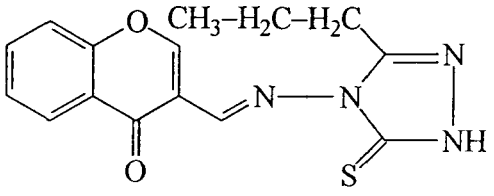


39

IR spectrum (Fig. 5) exhibit a broad band at 3451 cm^{-1} which is assigned to NH group. The ^1H NMR spectrum (Fig. 6) shows clear triplet and quartet for CH_3 and CH_2 protons at δ 1.33 and 2.80. Three aromatic protons H-6, H-7 and H-8 of chromone nucleus appear as two multiplets centred at δ 7.48-7.56 and 7.73-7.78. The H-5 proton is discernible as doublet at δ 8.33 (J=9Hz) whereas H-2 proton appears as sharp singlet at δ 8.70 which is quite a normal value. The proton of imine group again resonates at higher value δ 10.37 and NH protons appears as a broad singlet at δ 10.46 which is D_2O exchangeable.

3.1.3 The reaction of 1 with 4-amino-3-propyl-5-mercapto 1,2,4-triazole:

The mass (Fig.7) and IR spectra (Fig. 8) agree with the structure **40**.



40

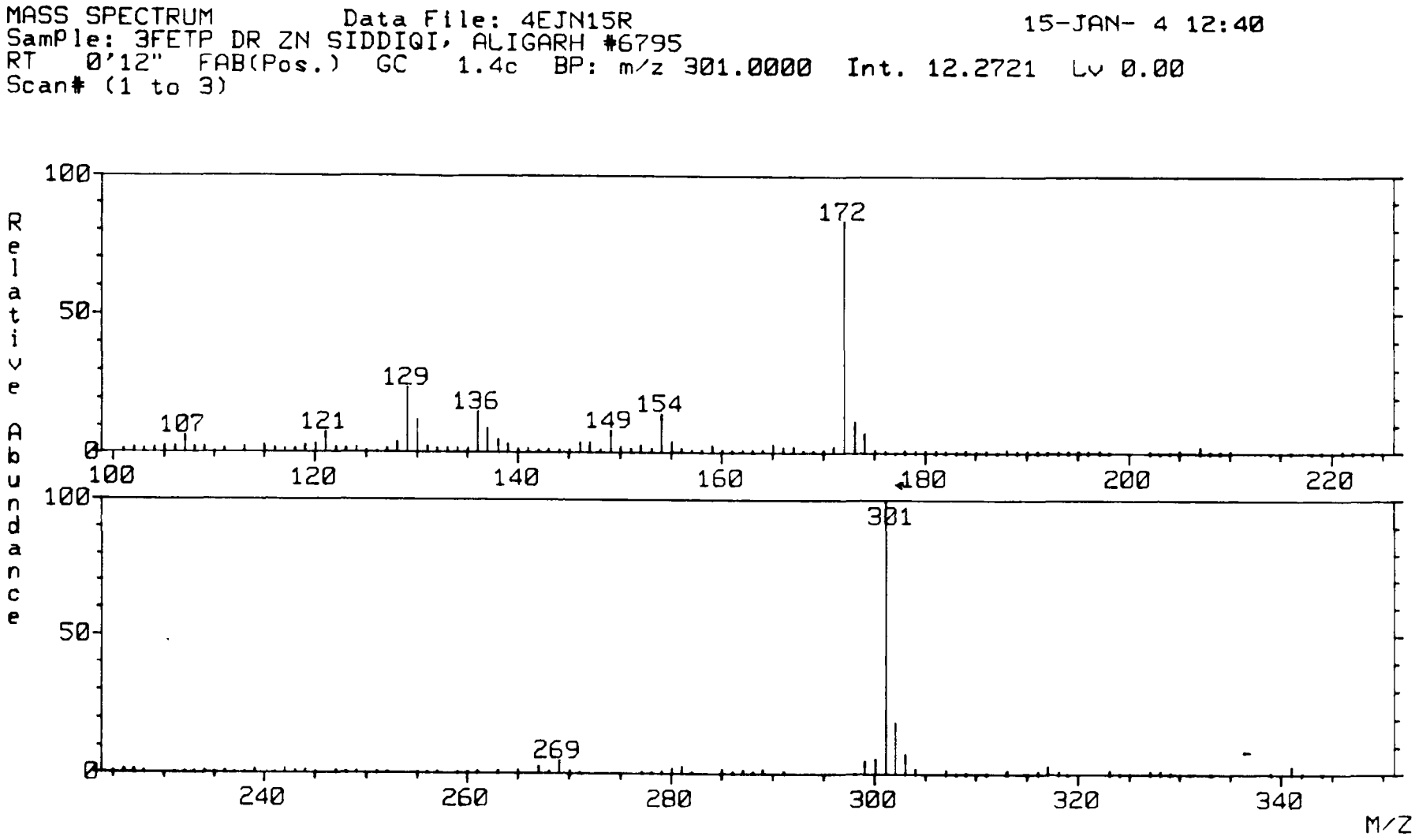

MASS SPECTRUM Data File: 4EJN15R 15-JAN- 4 12:40
Sample: 3FETP DR ZN SIDDIQI, ALIGARH #6795
RT 0'12" FAB(Pos.) GC 1.4c BP: m/z 301.0000 Int. 12.2721 Lv 0.00
Scan# (1 to 3)
Relative Abundance
M/Z
100
50
0
107
121
129
136
149
154
172
269
301
100
120
140
160
180
200
220
240
260
280
300
320
340

Fig. 4

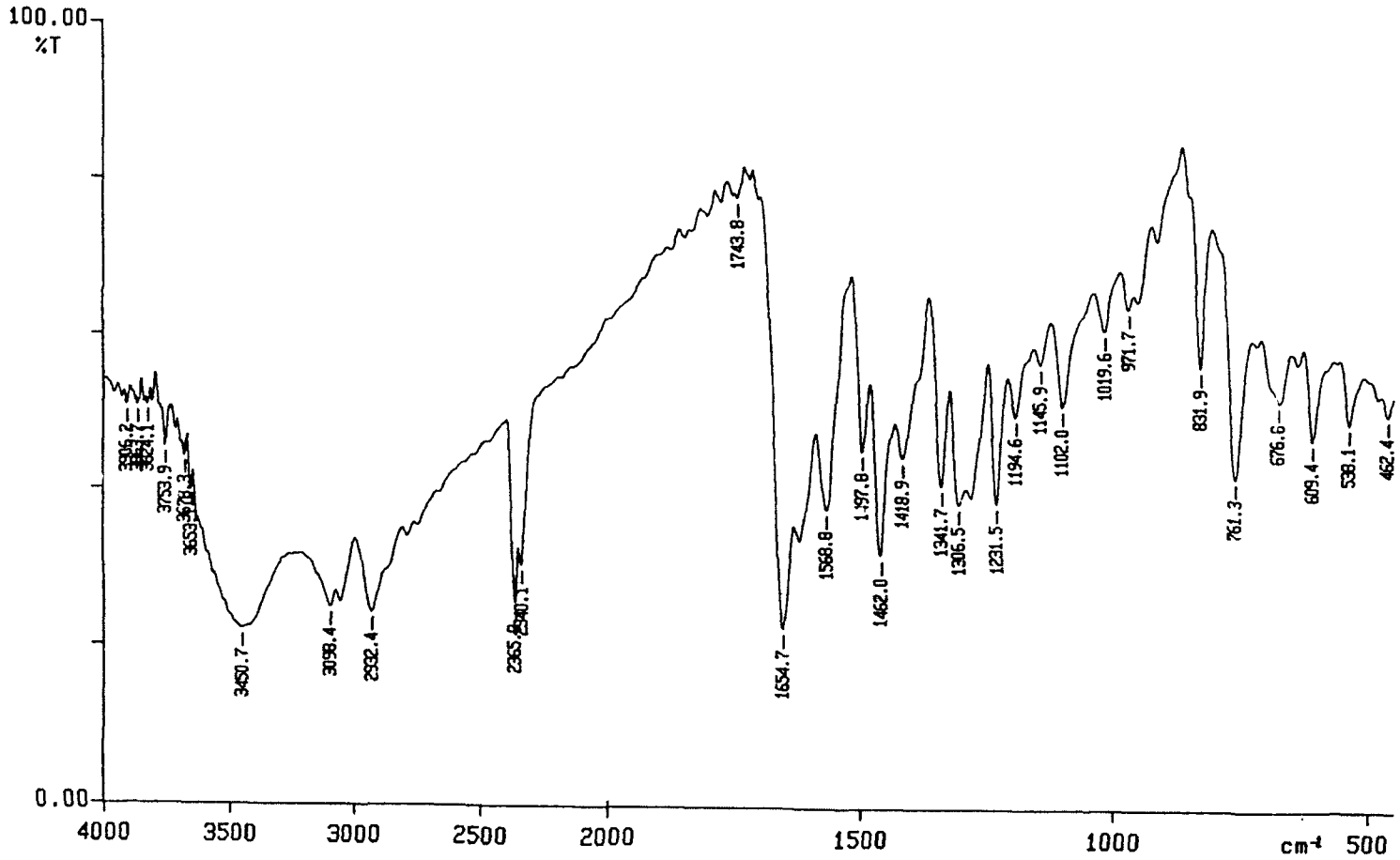

100.00
%T
0.00
4000
3500
3000
2500
2000
1500
1000
cm-1
500
3450.7
3098.4
2932.4
1743.8
1654.7
1568.8
1462.0
1418.9
1341.7
1306.5
1231.5
1194.6
1145.9
1102.0
1019.6
971.7
831.9
761.3
676.6
609.4
538.1
462.4
04/01/05 10:55 AH CODE-3FCTP
X: 4 scans, 4.0cm-1, flat, smooth, abex

Fig. 5

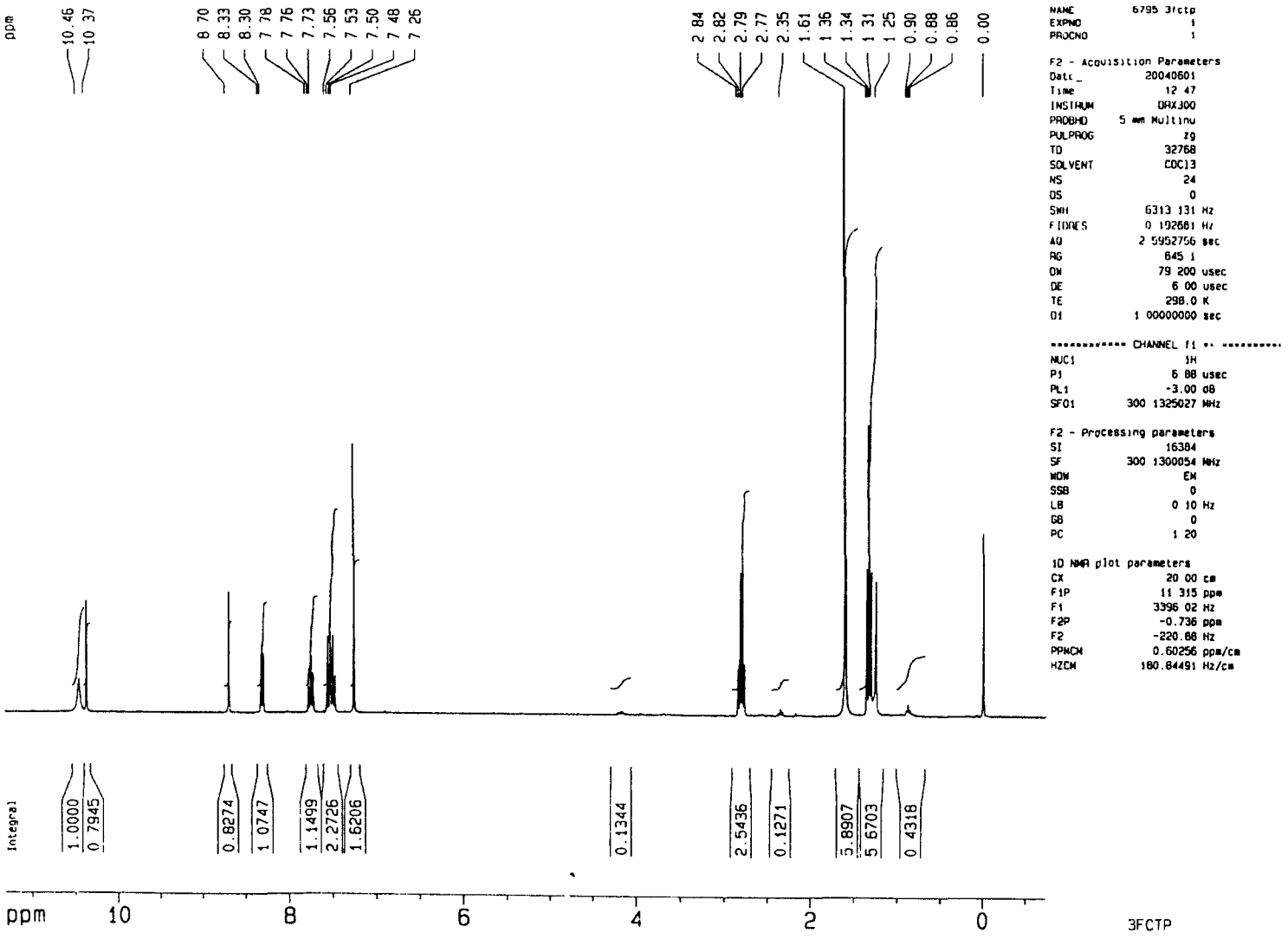

ppm
10.46
10.37
8.70
8.33
8.30
7.78
7.76
7.73
7.56
7.53
7.50
7.48
7.26
2.84
2.82
2.79
2.77
2.35
1.61
1.36
1.34
1.31
1.25
0.90
0.88
0.86
0.00
Integral
1.0000
0.7945
0.8274
1.0747
1.1499
2.2726
1.6206
0.1344
2.5436
0.1271
3.8907
5.6703
0.4318
ppm
10
8
6
4
2
0
3FCTP
NAME 6795 3fctp
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20040601
Time 12 47
INSTRUM DRX300
PROBHD 5 mm Multinu
PULPROG zg
TD 32768
SOLVENT CDCl3
NS 24
DS 0
SWH 6313 131 Hz
FIDRES 0 192681 Hz
AQ 2 5952756 sec
RG 645 1
DW 79 200 usec
DE 6 00 usec
TE 298.0 K
D1 1 00000000 sec
CHANNEL f1
NUC1 1H
P1 6 88 usec
PL1 -3.00 dB
SFO1 300 1325027 MHz
F2 - Processing parameters
SI 16384
SF 300 1300054 MHz
WDW EM
SSB 0
LB 0 10 Hz
GB 0
PC 1 20
1D NMR plot parameters
CX 20 00 cm
F1P 11 315 ppm
F1 3396 02 Hz
F2P -0.736 ppm
F2 -220.68 Hz
PPMCM 0.60256 ppm/cm
HZCM 180.84491 Hz/cm

Fig. 6

[Mass Spectrum]
Data : 4ENOV29120 Date : 29-Nov-2004 11:31
Sample: 3FCTB DR Z N SIDDIQUI AMU ALIGARH #7876
Note :
Inlet : Direct Ion Mode : FAB+
Spectrum Type : Normal Ion [MF-Linear]
RT : 0.49 min Scan# : (4,6)
BP : m/z 172.0000 Int. : 43.11
Output m/z range : 89.9852 to 507.5265 Cut Level : 0.00 %

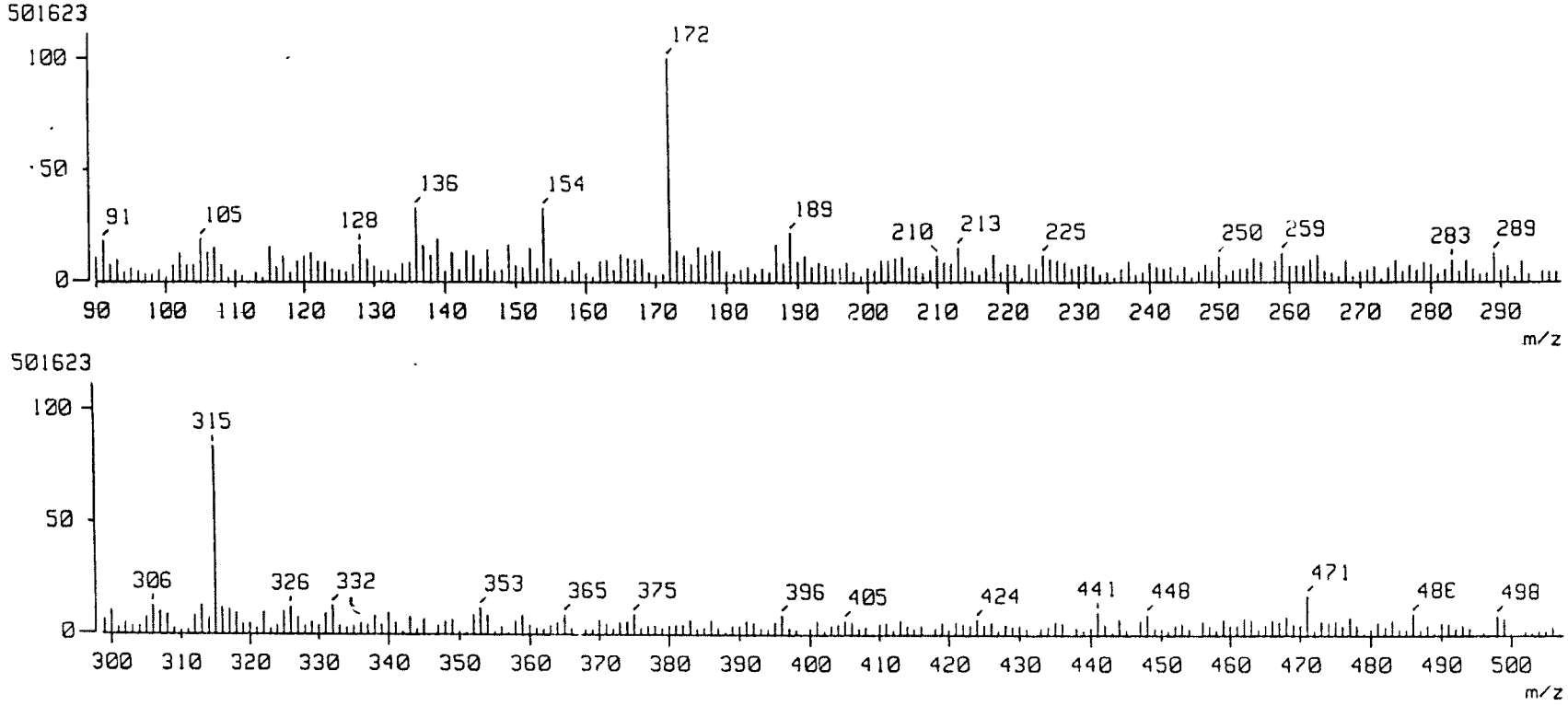


Fig. 7

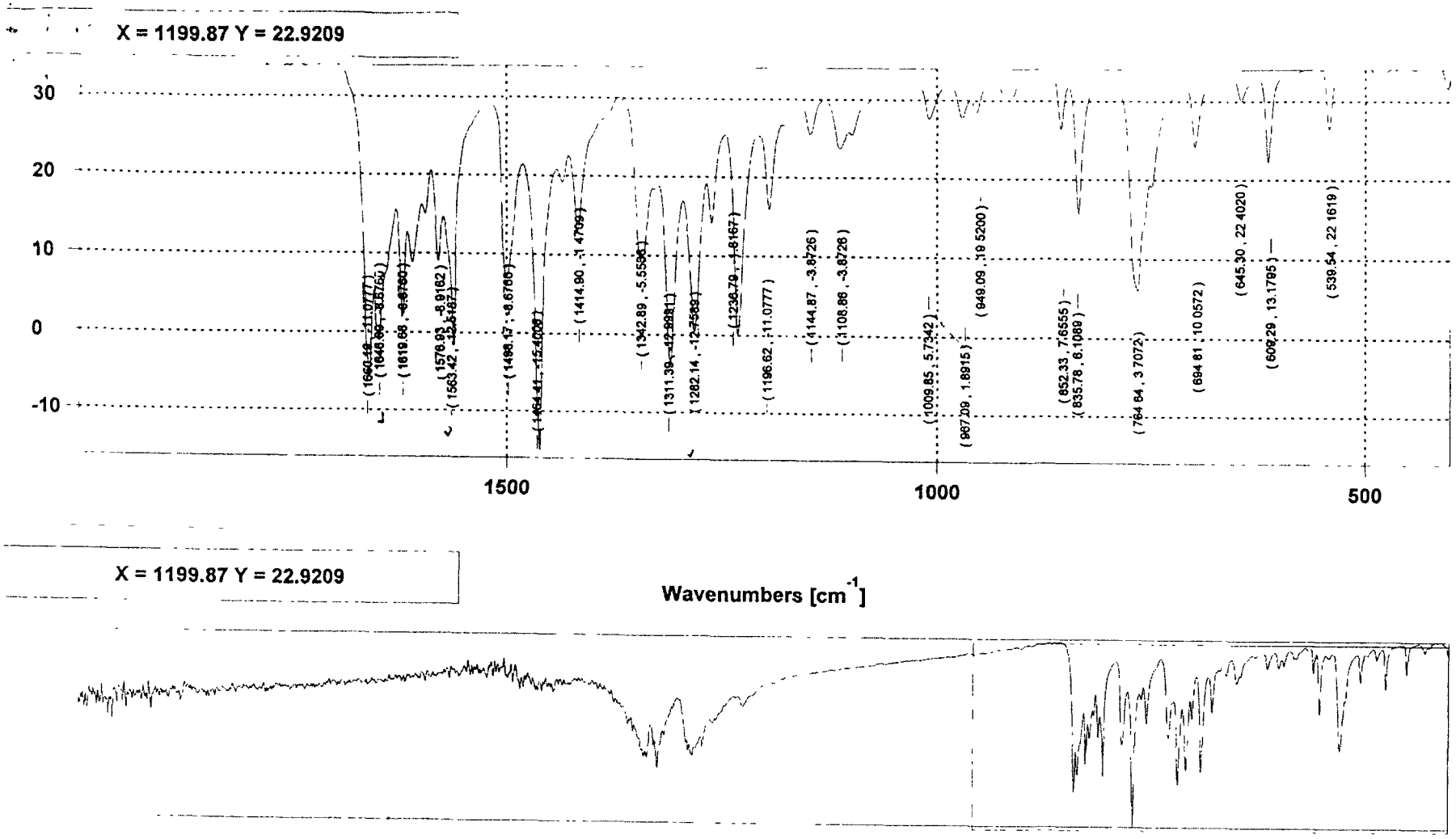

X = 1199.87 Y = 22.9209
30
20
10
0
-10
1500
1000
500
X = 1199.87 Y = 22.9209
Wavenumbers [cm-1]

Fig. 8

The ^{1}H NMR spectrum (Fig. 9) displays signals for CH_3, CH_2, CH_2 protons as triplet at δ 1.04, multiplet at δ 1.81 and triplet at δ 2.77 respectively. Besides it, H-5 proton appears as double doublet at δ 8.32 and H-2 proton as a sharp singlet at δ 8.71. Another sharp singlet at δ 10.35 and a broad singlet at δ 11.22 which is exchangeable with D_2O, has been assigned to imine and NH protons respectively.

3.1.4 Cyclization of Schiff's bases, 38-40:

Cyclization of Schiff's bases take place by refluxing in a high boiling solvent[41]. In order to get cyclized product Schiff's, bases **38-40,** were refluxed in nitrobenzene for sometime. After removal of solvent under reduced pressure, the reaction mixture was chromatographed over silica gel. Elution of the column with benzene-petrol with varying ratio afforded two compounds, 3FCTNB as a major product and 3FCTPNB as a minor one.

3FCTNB:

The IR spectrum (Fig. 10) of the compound shows a slightly broad and strong absorption band at 1654 cm^{-1} showing the presence of more than one chromone carbonyl group. The ^{1}H NMR (Fig. 11) of the compound does not show any methyl signal in the region δ 2.2 to 2.4. This rules out the possibility of a structure **41** for the compound which is also not compatible with the mass spectrum (Fig. 12).

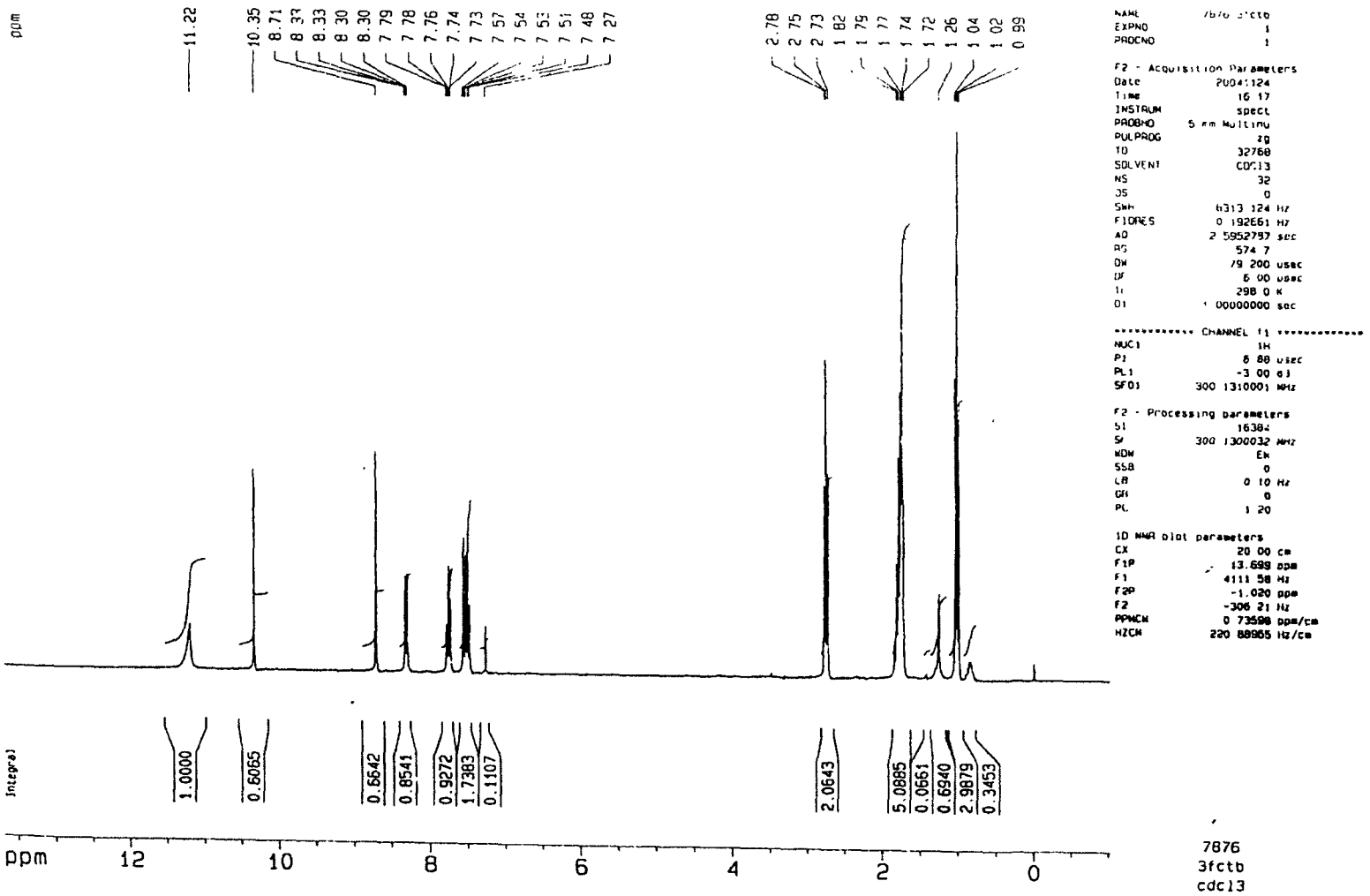

ppm
11.22
10.35
8.71
8.33
8.33
8.30
8.30
7.79
7.78
7.76
7.74
7.73
7.57
7.54
7.53
7.51
7.48
7.27
2.78
2.75
2.73
1.82
1.79
1.77
1.74
1.72
1.26
1.04
1.02
0.99
Integral
1.0000
0.6065
0.6642
0.8541
0.9272
1.7383
0.1107
2.0643
5.0885
0.0661
0.6940
2.9879
0.3453
ppm
12
10
8
6
4
2
0
NAME 7876 3fctb
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date 20041124
Time 16 17
INSTRUM spect
PROBHD 5 mm Multinu
PULPROG zg
TD 32768
SOLVENT CDCl3
NS 32
DS 0
SWH 6313 124 Hz
FIDRES 0 192661 Hz
AQ 2 5952797 sec
RG 574 7
DW 79 200 usec
DE 6 00 usec
TE 298 0 K
D1 1 00000000 sec
CHANNEL f1
NUC1 1H
P1 8 88 usec
PL1 -3 00 dB
SFO1 300 1310001 MHz
F2 - Processing parameters
SI 16384
SF 300 1300032 MHz
WDW EM
SSB 0
LB 0 10 Hz
GB 0
PC 1 20
1D NMR plot parameters
CX 20 00 cm
F1P 13.698 ppm
F1 4111 58 Hz
F2P -1.020 ppm
F2 -306 21 Hz
PPMCM 0 73598 ppm/cm
HZCM 220 88965 Hz/cm
7876
3fctb
cdcl3

Fig. 9

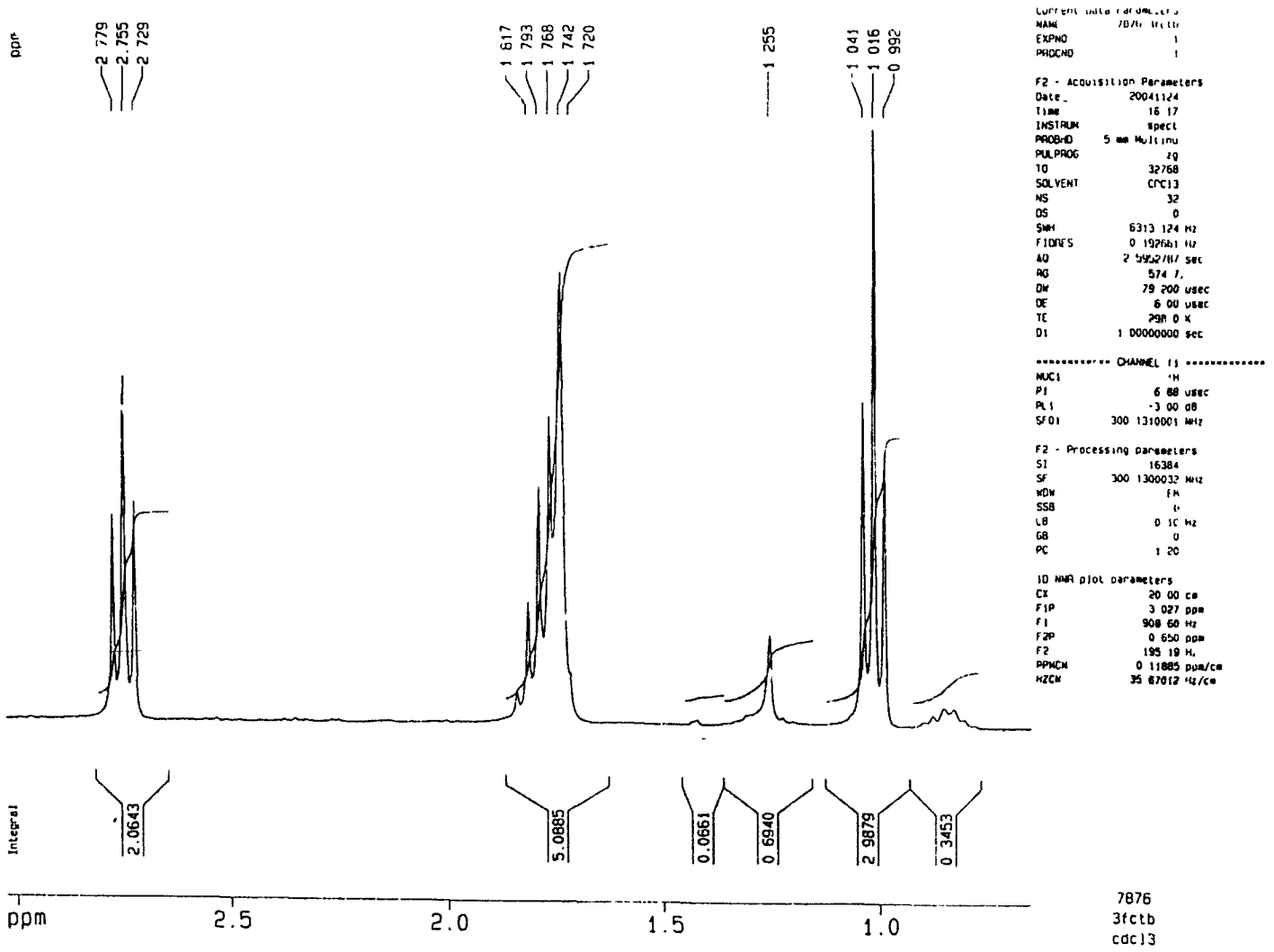


Fig. 9 (Expanded form)

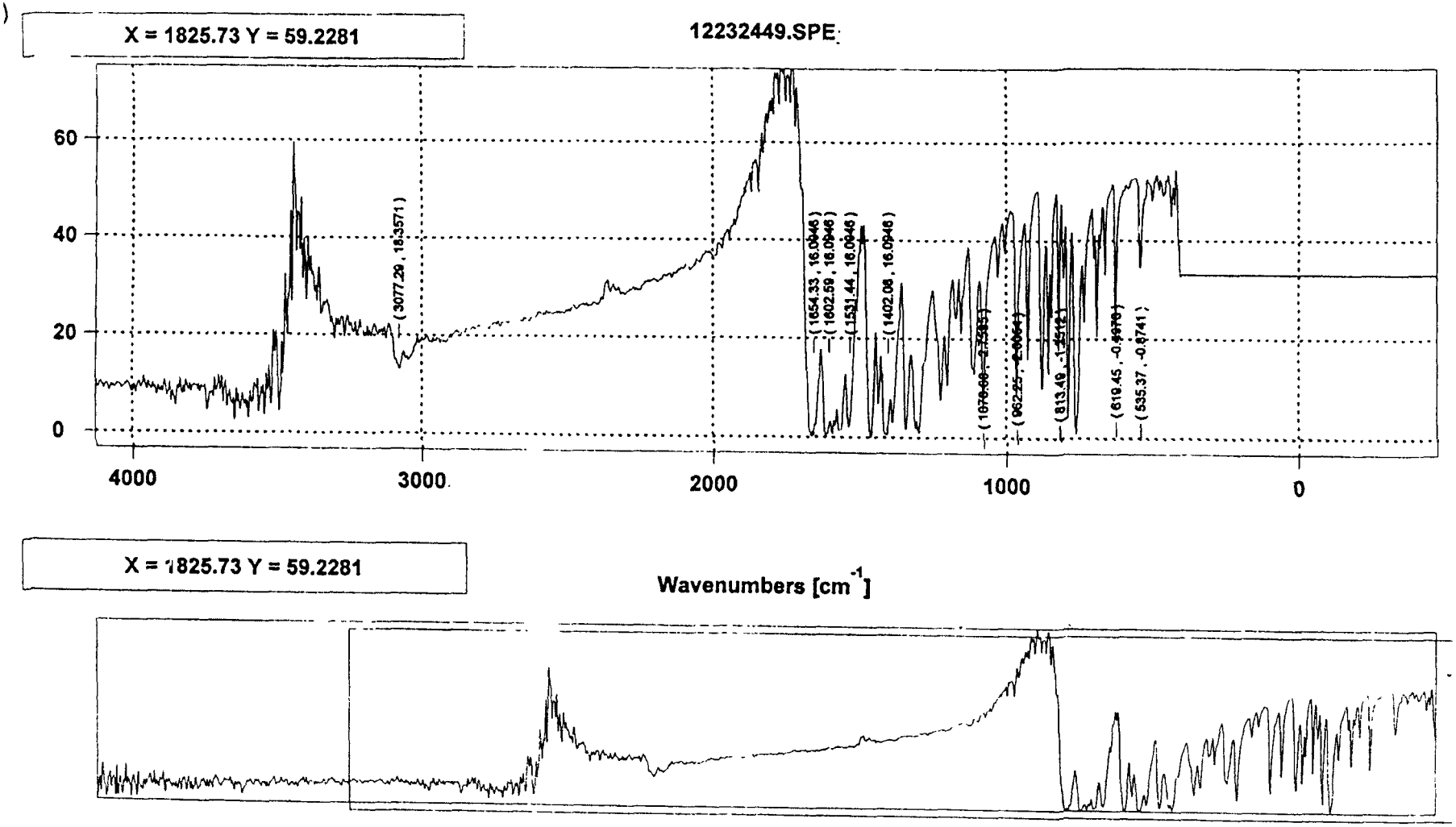

X = 1825.73 Y = 59.2281
12232449.SPE
60
40
20
0
4000
3000
2000
1000
0
X = 1825.73 Y = 59.2281
Wavenumbers [cm-1]

Fig. 10

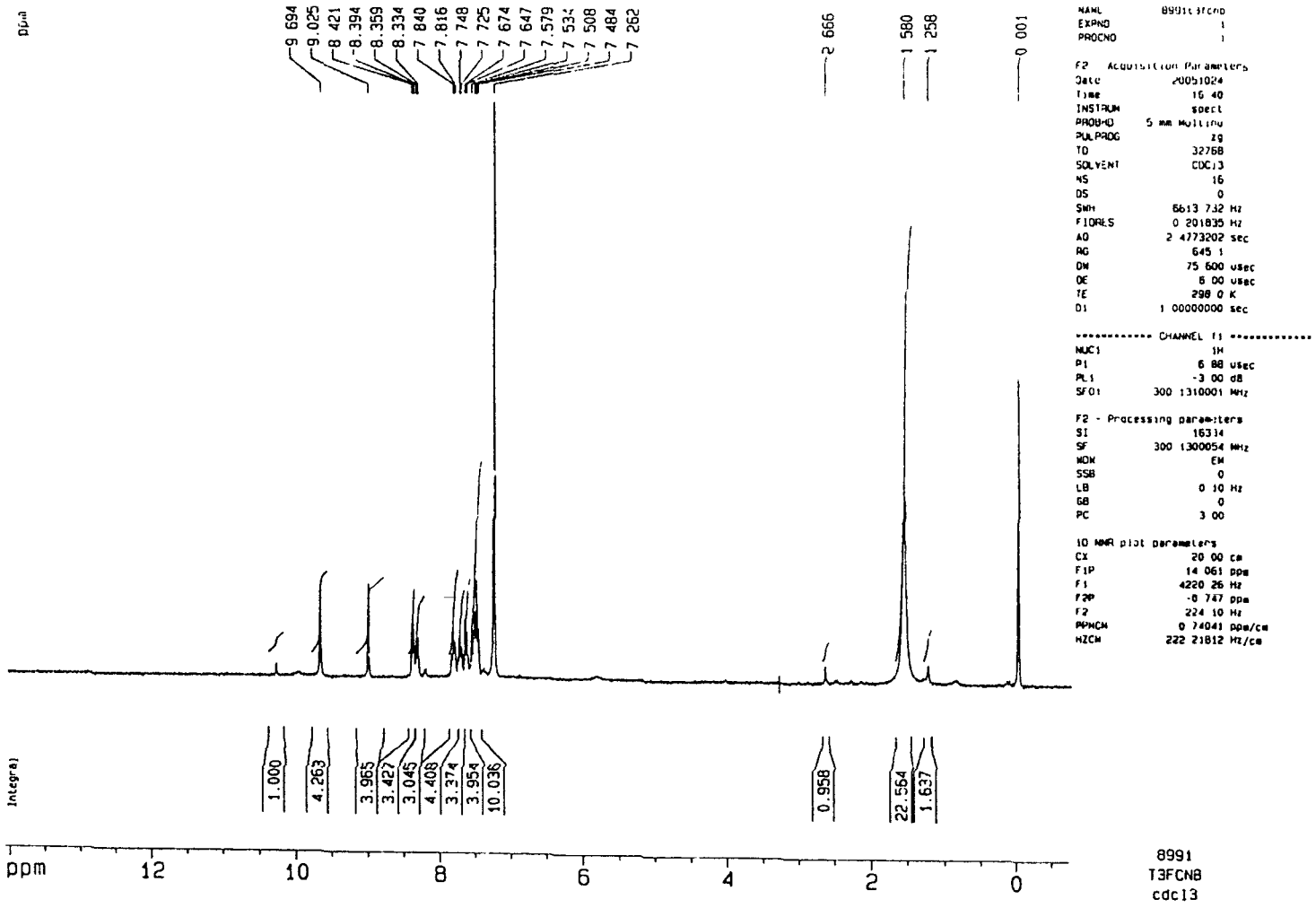

9.694
9.025
8.421
8.394
8.359
8.334
7.840
7.816
7.748
7.725
7.674
7.647
7.579
7.534
7.508
7.484
7.262
2.666
1.580
1.258
0.001
Integral
1.000
4.263
3.965
3.427
3.045
4.408
3.374
3.954
10.036
0.958
22.564
1.637
ppm
12
10
8
6
4
2
0
8991
T3FCN8
cdcl3

Fig. 11

[Mass Spectrum]
Data : 5EJUNE17466 Date : 17-Jun-2005 09:24
Sample: T3FCNB DR Z N SIDDIQUE ALIGARH #8633
Note : -
Inlet : Direct Ion Mode : FAB+
Spectrum Type : Normal Ion [MF-Linear]
RT : 0.12 min Scan# : (1,4)
BP : m/z 343.0000 Int. : 93.52
Output m/z range : 50.0000 to 609.7923 Cut Level : 0.00 %

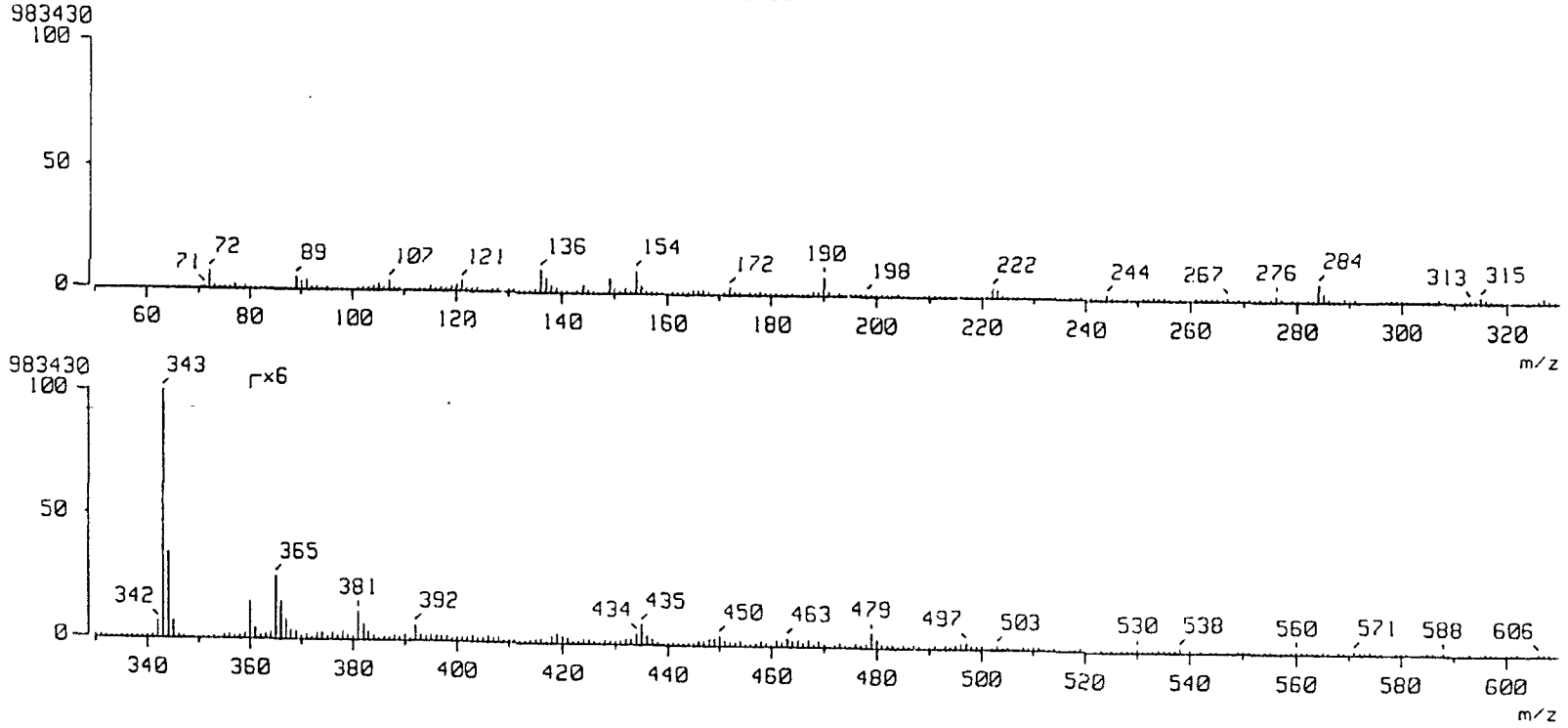
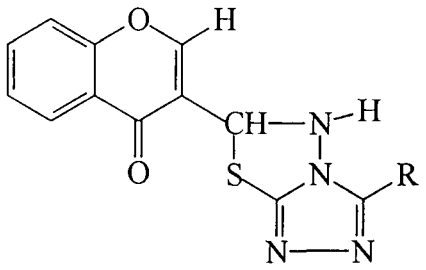
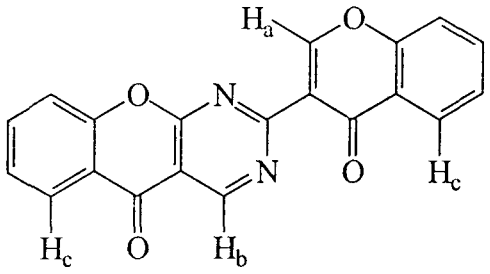


Fig. 12



41

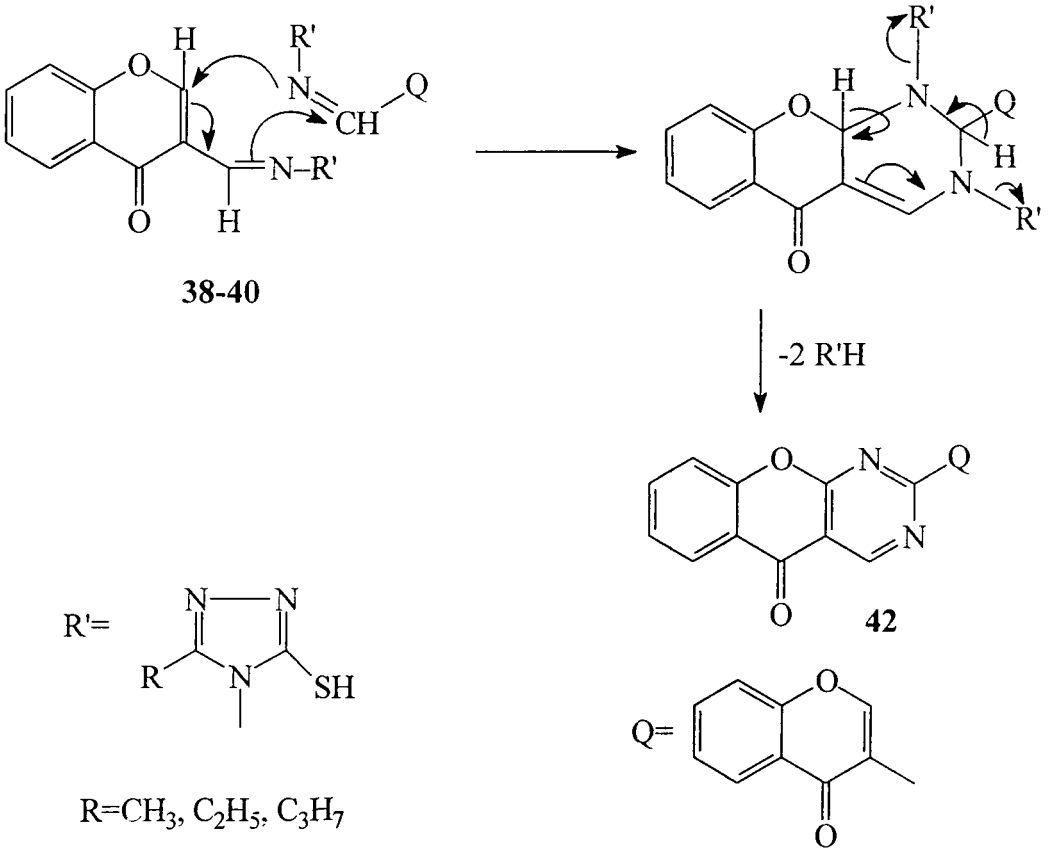
The ^{1}HNMR spectrum clearly shows the characteristic C-5 signals of chromone units as a doublet integrating for two protons at δ 8.24. This indicates the presence of two chromone nuclei in the compound. In addition to this, the spectrum also shows two sharp singlets, each integrating for one proton at δ 9.14 and 9.58. The former can be assigned to C-2 proton (H_a) of the chromone nucleus whereas the later at higher value can be assigned to a proton which is under the influence of some electron withdrawing group. On the basis of these structural features structure **42** may be assigned to the compound.



42

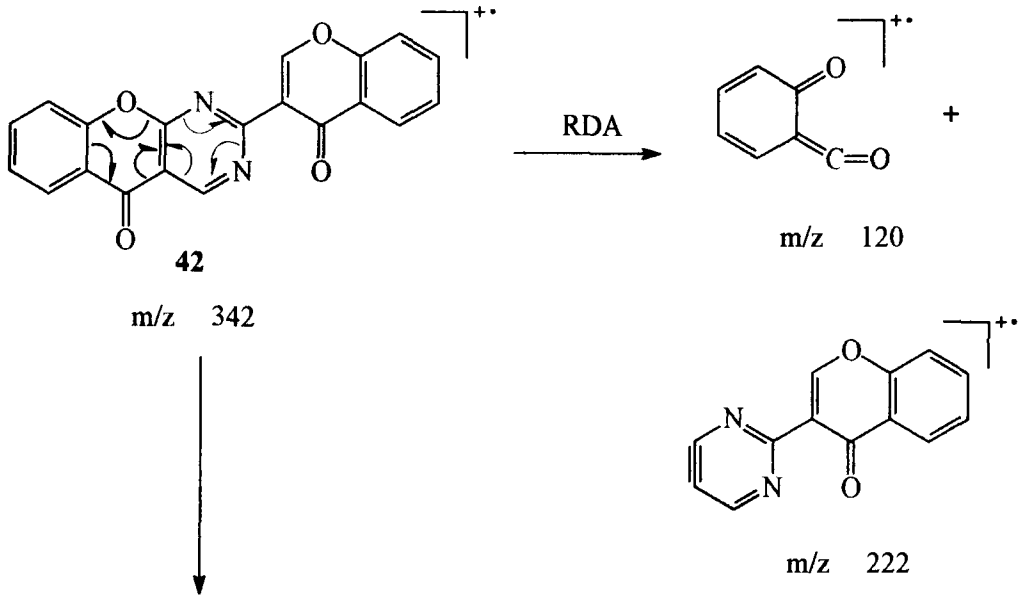
On the basis of structure **42** the singlet at δ 9.58 is now assigned to the proton which is *peri* to oxygen (H_b) and experiences deshielding effect of electron withdrawing >C=O group of chromone unit. Since structure **42** does not have any alkyl group, it seems that the compound

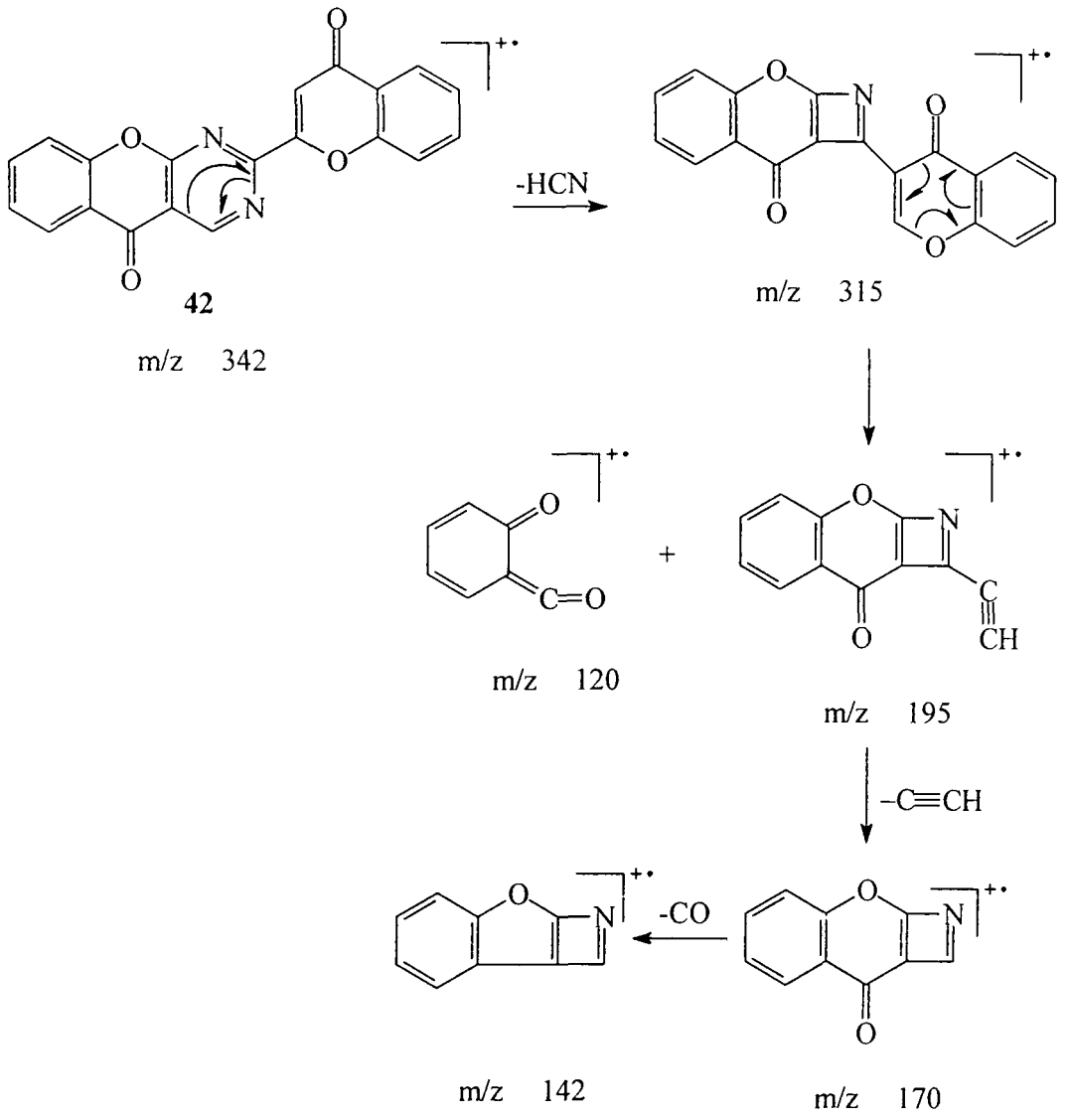
42 on refluxing in nitrobenzene, is obtained as a result of dimerisation of the Schiff's bases **38-40** involving (4+2) cycloaddition reaction with the explusion of triazole moiety as shown in Scheme 13.



Scheme 13

The structure **42** is also in confirmity with its mass spectrum (Fig. 12) which shows M^+ at m/z 343. (M+1 peak). Elimination of HCN or CN groups is a common feature in the mass spectrum of pyrimidines/pyridazines[42]. Molecular ion peak, therefore, gives peak at m/z 315 by removal HCN. The other relevant peaks are obtained as a result of break down of the compound as shown in Scheme 14.


RDA
+
C=O
42
m/z 342
m/z 120
m/z 222
+ HCN +
N≡C
m/z 144
m/z 171
–CO
m/z 116



Scheme 14

3FCTPNB:

The compound shows M^+ at m/z 343 (M+1 peak) in its mass spectrum (Fig. 13) Since it has the same molecular ion peak as that of **42,** it was thought that it is the same compound but repeated TLC, Co TLC and melting point confirmed that two compounds are different. The IR spectrum (Fig. 14) of the compound shows a broad and strong absorption band at

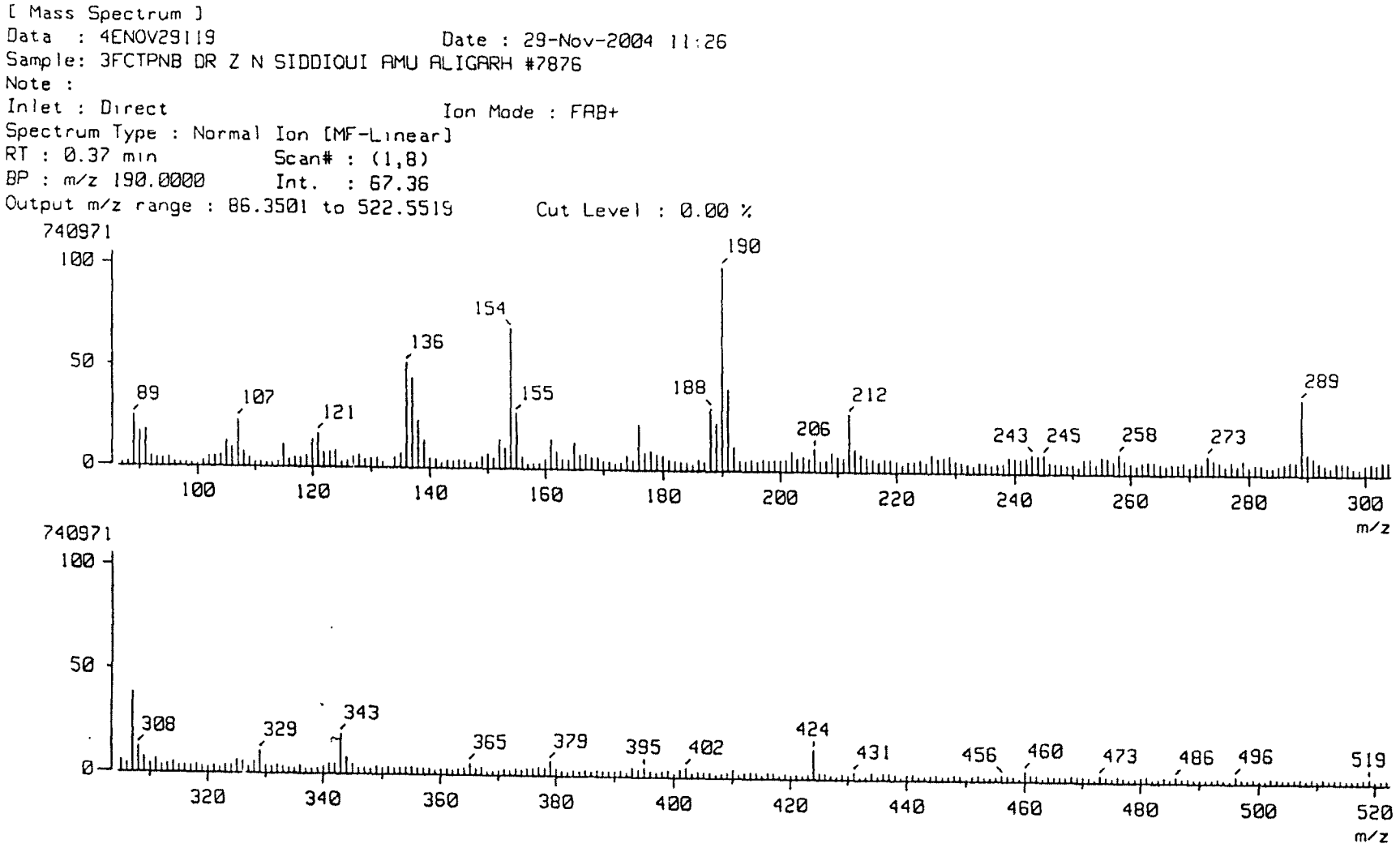

[Mass Spectrum]
Data : 4ENOV29119 Date : 29-Nov-2004 11:26
Sample: 3FCTPNB DR Z N SIDDIQUI AMU ALIGARH #7876
Note :
Inlet : Direct Ion Mode : FAB+
Spectrum Type : Normal Ion [MF-Linear]
RT : 0.37 min Scan# : (1,8)
BP : m/z 190.0000 Int. : 67.36
Output m/z range : 86.3501 to 522.5519 Cut Level : 0.00 %
740971
100
50
0
89
107
121
136
154
155
188
190
206
212
243
245
258
273
289
100
120
140
160
180
200
220
240
260
280
300
m/z
740971
100
50
0
308
329
343
365
379
395
402
424
431
456
460
473
486
496
519
320
340
360
380
400
420
440
460
480
500
520
m/z

Fig. 13

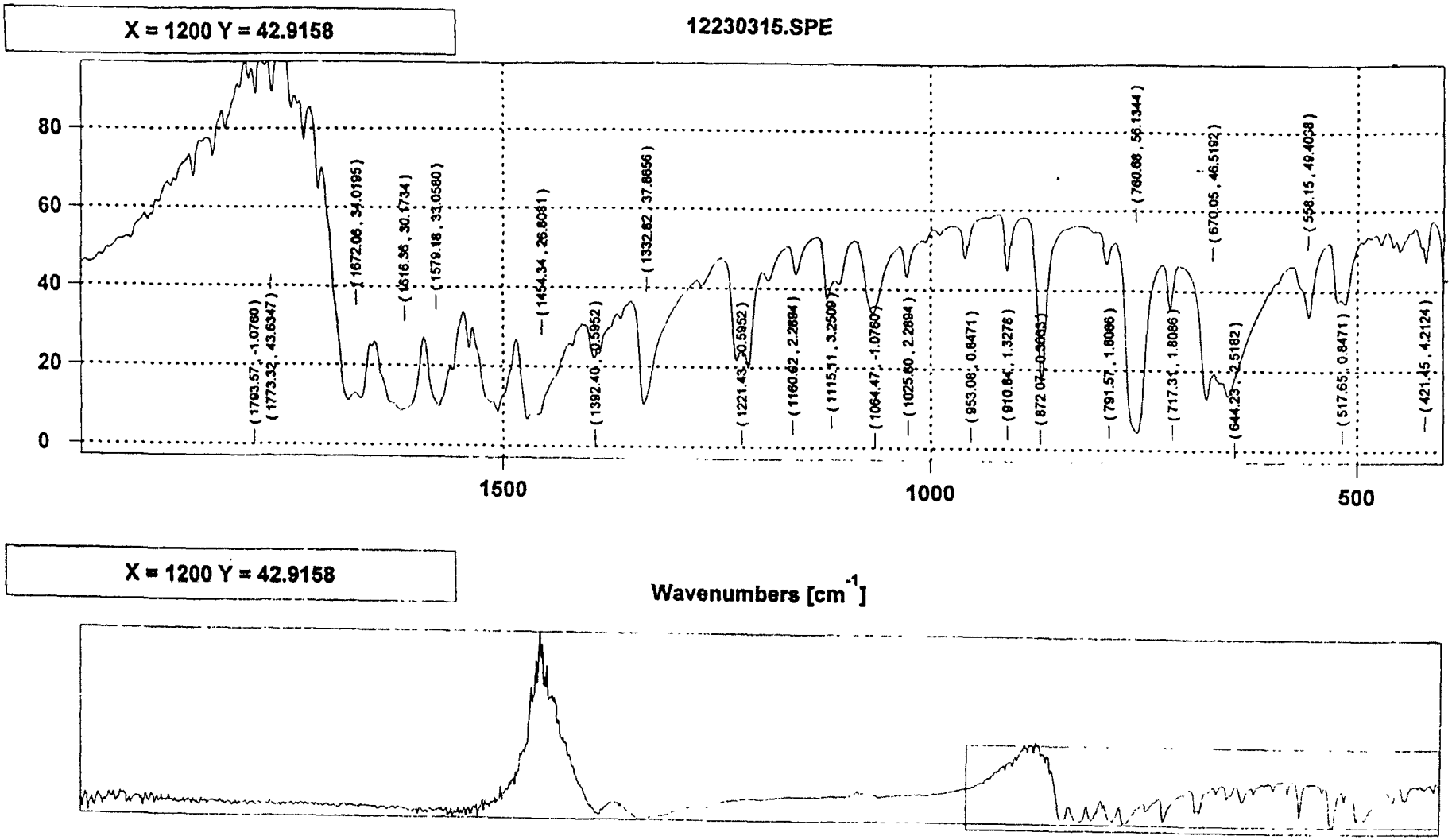
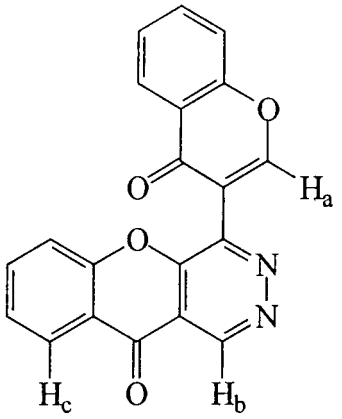

X = 1200 Y = 42.9158
12230315.SPE
80
60
40
20
0
1500
1000
500
(1793.57 , -1.0760)
(1773.32 , 43.6347)
(1672.08 , 34.0195)
(1616.36 , 30.1734)
(1579.18 , 33.0580)
(1454.34 , 26.8081)
(1392.40 , 0.5952)
(1332.82 , 37.8656)
(1221.43 , 20.5952)
(1160.62 , 2.2884)
(1115.11 , 3.2509)
(1064.47 , -1.0760)
(1025.80 , 2.2884)
(953.08 , 0.8471)
(910.84 , 1.3278)
(872.07 , 0.3603)
(791.57 , 1.8086)
(760.88 , 56.1344)
(717.31 , 1.8086)
(670.05 , 46.5192)
(644.23 , 2.5182)
(558.15 , 49.4038)
(517.65 , 0.8471)
(421.45 , 4.2124)
X = 1200 Y = 42.9158
Wavenumbers [cm-1]

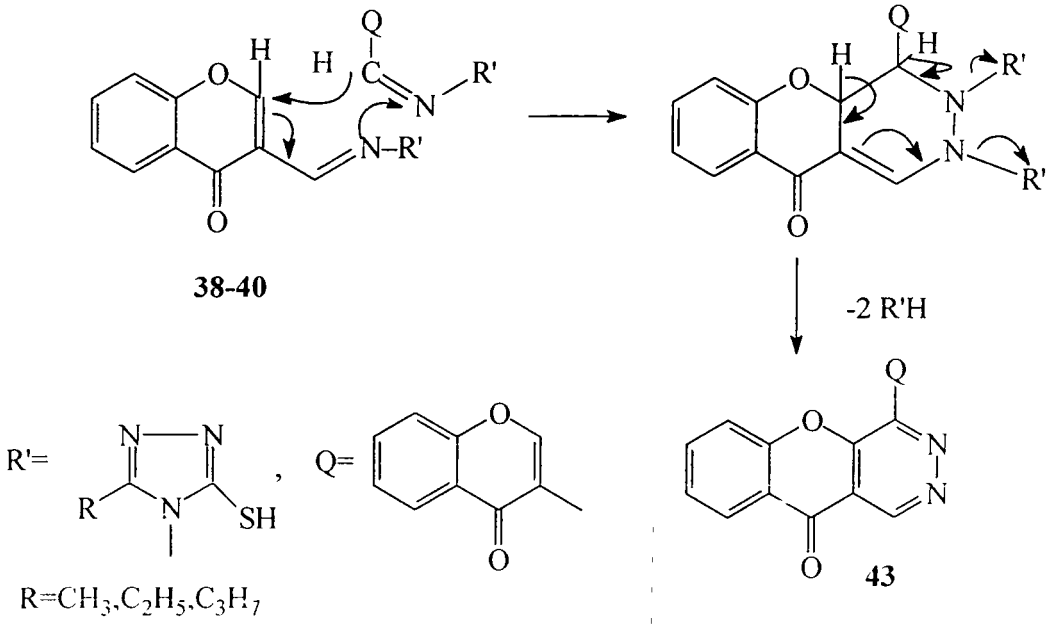
Fig. 14

1672 cm^{-1} indicating the presence of chromone carbonyl group in the compound. The nmr spectrum (Fig. 15) exhibits a sharp singlet integrating for one proton at δ 10.30 and a doublet of C-5 proton (H_c) of chromone nucleus at δ 8.24 (J=7.8 Hz). Another doublet is situated at δ 7.63 which may be assigned to C-5 proton of other chromone nucleus. Since the molecular weight of the compound is the same, it appears that the compound is a functional/positional isomer of **42**.



43

In the light of these spectral features structure **43** can be assigned to the compound the formation of which has been shown in Scheme 15.



Scheme 15

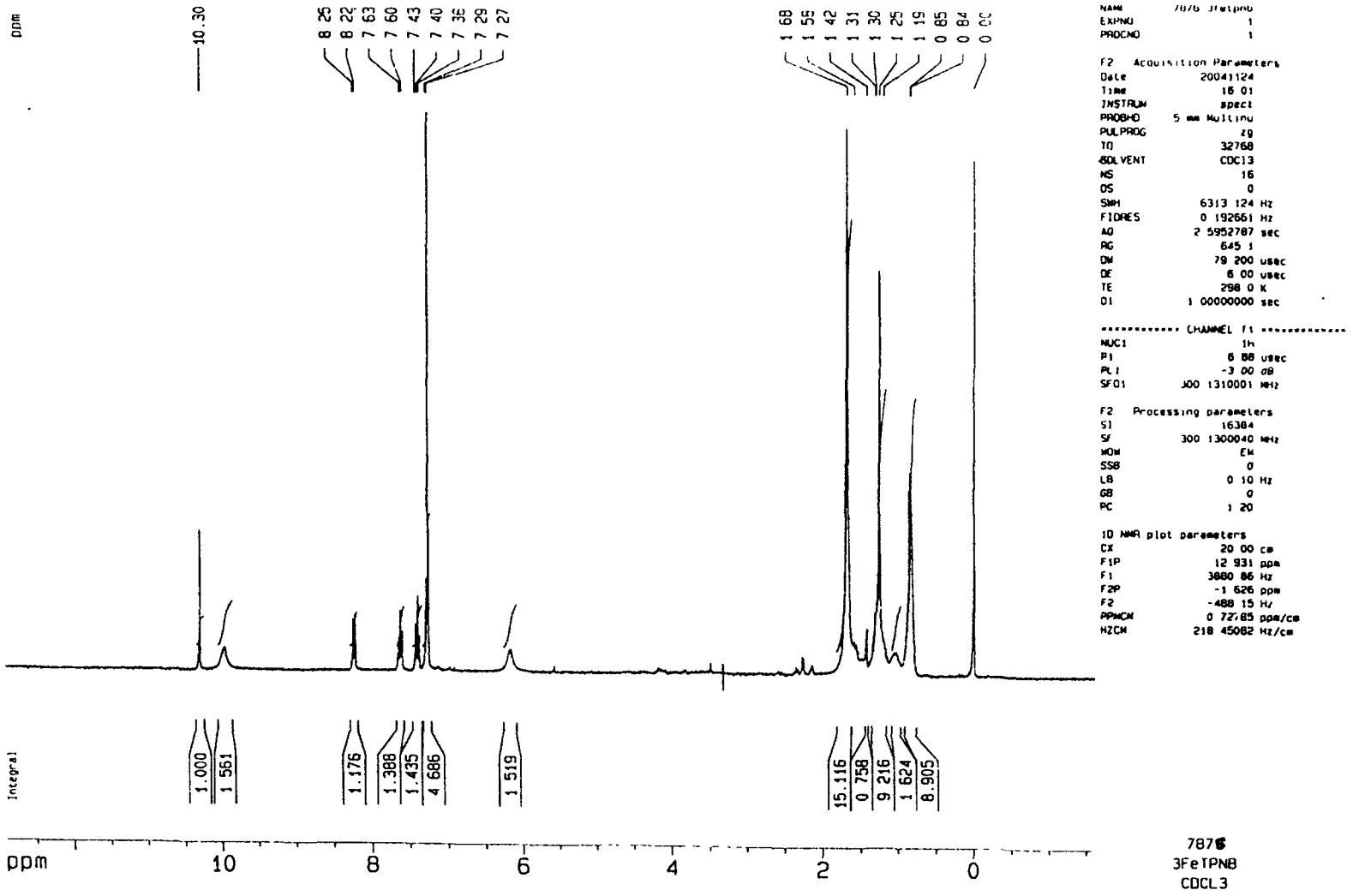
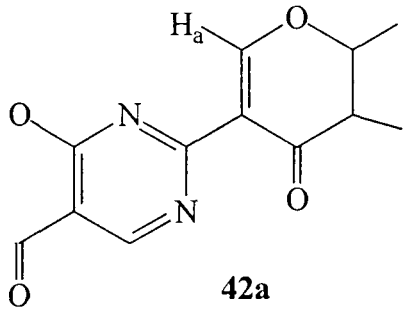
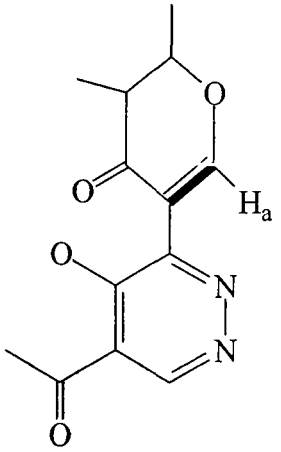

ppm
10.30
8.25
8.22
7.63
7.60
7.43
7.40
7.38
7.29
7.27
1.68
1.55
1.42
1.31
1.30
1.25
1.19
0.85
0.84
Integral
1.000
1.561
1.176
1.388
1.435
4.686
1.519
15.116
0.758
9.216
1.624
8.905
ppm
10
8
6
4
2
0
7876
3FeTPNB
CDCL3
EXPNO 1
PROCNO 1
F2 Acquisition Parameters
Date 20041124
Time 16 01
INSTRUM spect
PROBHD 5 mm Multinu
PULPROG zg
TD 32768
SOLVENT CDCl3
NS 16
DS 0
SWH 6313 124 Hz
FIDRES 0 192661 Hz
AQ 2 5952787 sec
RG 645 1
DW 79 200 usec
DE 6 00 usec
TE 298 0 K
D1 1 00000000 sec
CHANNEL f1
NUC1 1h
P1 6 88 usec
PL1 -3 00 dB
SFO1 300 1310001 MHz
F2 Processing parameters
SI 16384
SF 300 1300040 MHz
WDW EM
SSB 0
LB 0 10 Hz
GB 0
PC 1 20
1D NMR plot parameters
CX 20 00 cm
F1P 12 931 ppm
F1 3880 86 Hz
F2P -1 626 ppm
F2 -488 15 Hz
PPMCM 0 72785 ppm/cm
HZCM 218 45082 Hz/cm

Fig. 15

The only ambiguous part of the nmr spectrum is the presence of a slightly broad singlet integrating for one proton at δ 10.0 which is assigned to H_a proton of chromone unit. The broadening of singlet may be due to restricted rotation of chromone unit in compound **43** which is not noticed for the same proton due to more freedom to rotation of chromone unit in compound **42** as shown in structures **42a** and **43a**



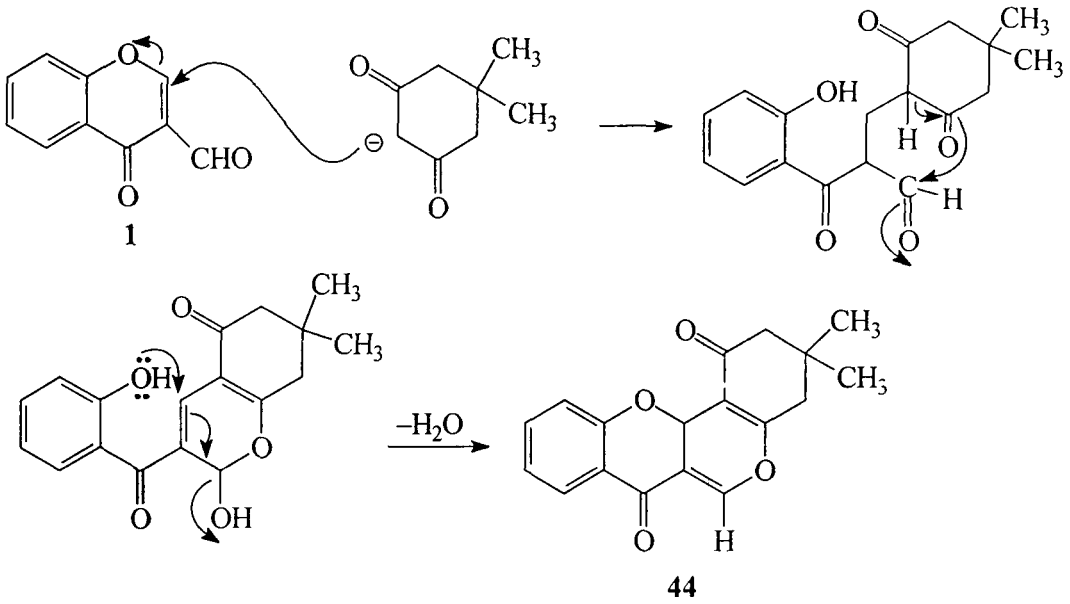
42a



43a

3.2 The reaction of 3-formylchromone with dimedone:

3-Formylchromone undergoes ring opening reaction with active methylene compounds such ethylacetoacetate to give o-hydroxyacetophenone derivatives[11]. It seemed, therefore interesting what course would be followed if the reaction is carried out with active methylene compound, dimedone. Thus, one would expect **44** from the reaction of **1** and dimedone. The mechanism leading to **44** involves the sequence of reactions shows below.



Scheme 16

The mass spectrum (Fig. 16) of the product agrees with the assigned structure showing M^+ at 295. Since the compound does not have any nitrogen in its structure, therefore, m/z 296 was considered as molecular ion peak. The IR spectrum (Fig. 17) shows slightly broad and strong absorption band at 1630 cm^{-1} indicating the presence of more than one carbonyl groups. Thus, if **44** is the structure for the compound, then IR spectrum should display the band at 1660-1670 cm^{-1} rather than at 1630 cm^{-1}, which is the absorption region for chromone carbonyl group. The NMR, spectrum (Fig. 18) shows a sharp singlet integrating for six protons at δ 0.98 indicating the presence of two methyl groups in the compound. However, two methylene groups which usually appear as singlet or sometimes multiplet at δ 2.2-2.5 are not visible in the spectrum because of presence of water and DMSO peaks in the same region. In the NMR spectrum, the ortho coupled

[Mass Spectrum]
Data : 5ESEP23609 Date : 23-Sep-2005 11:12
Sample: 3FED DR ZEBA N SIDDIQUI,ALIGARH #8935
Note : -
Inlet : Direct Ion Mode : FAB+
Spectrum Type : Normal Ion [MF-Linear]
RT : 0.12 min Scan# : (1,4)
BP : m/z 154.0000 Int. : 100.00
Output m/z range : 97.2552 to 551.6320 Cut Level : 0.00 %

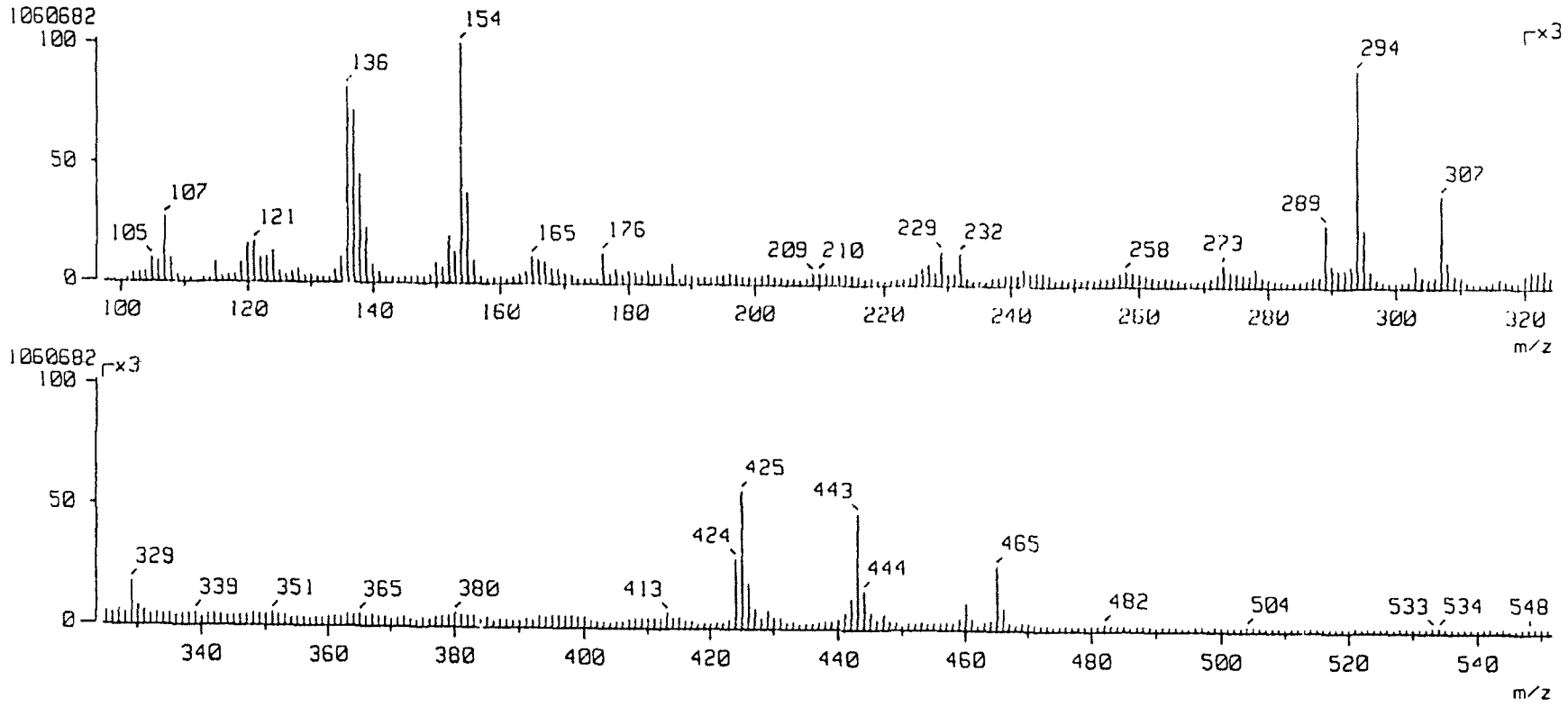


Fig. 16

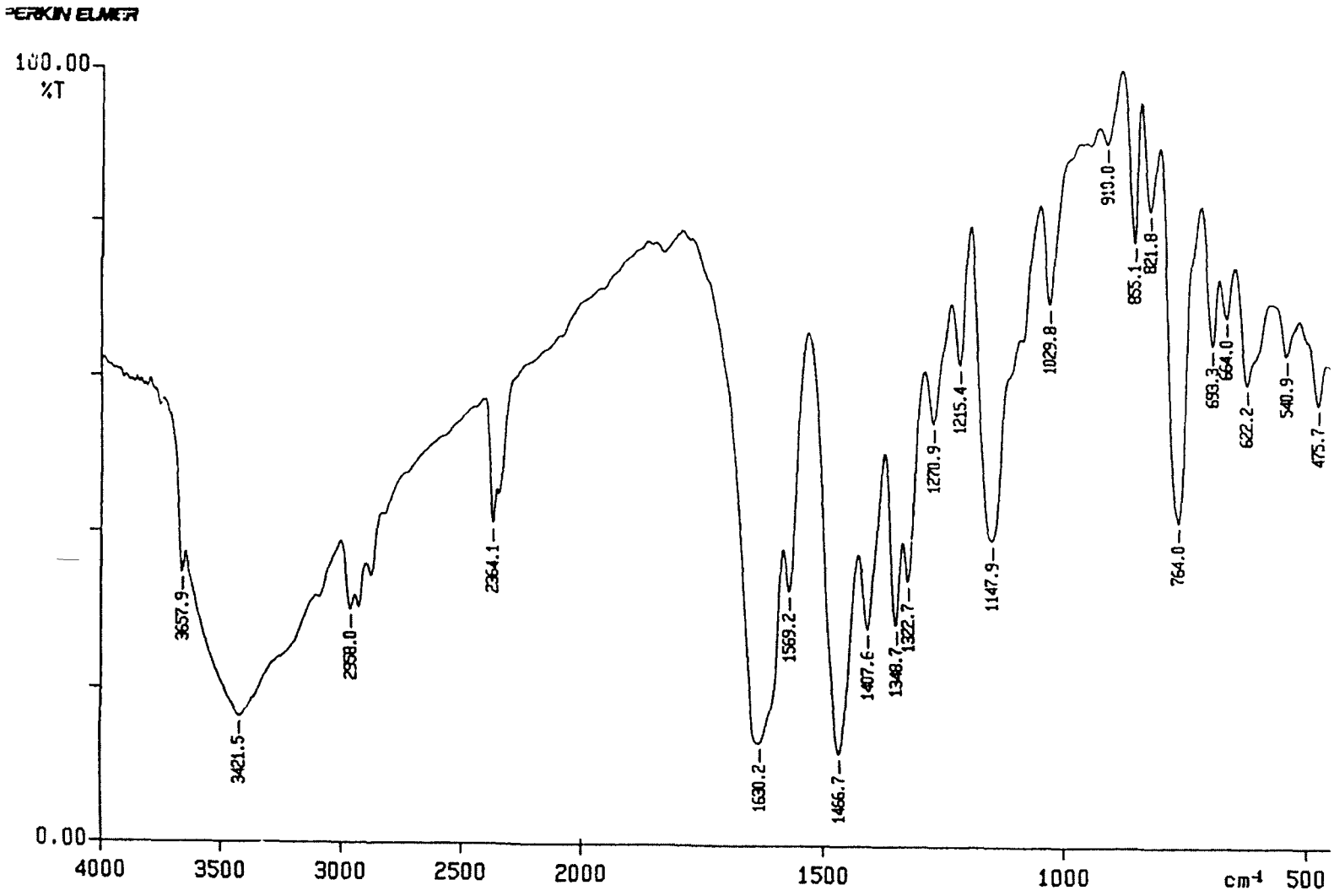

PERKIN ELMER
100.00
%T
0.00
4000
3500
3000
2500
2000
1500
1000
cm-1
500
3657.9
3421.5
2938.0
2364.1
1630.2
1569.2
1466.7
1407.6
1348.7
1322.7
1270.9
1215.4
1147.9
1029.8
910.0
855.1
821.8
764.0
693.3
664.0
622.2
540.9
475.7

Fig. 17

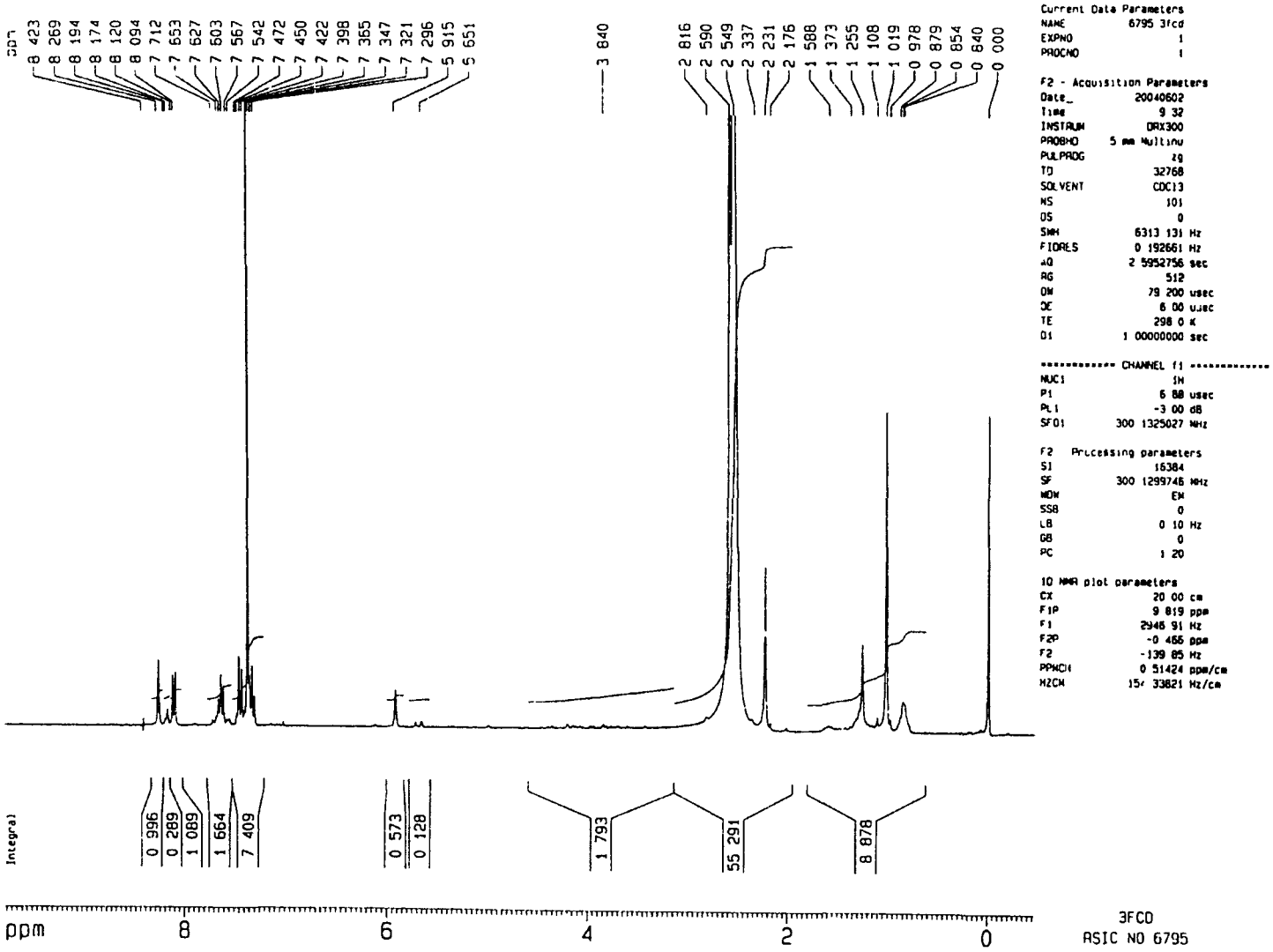
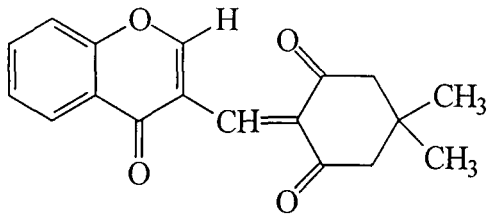

8.423
8.269
8.194
8.174
8.120
8.094
7.712
7.653
7.627
7.603
7.567
7.542
7.472
7.450
7.422
7.398
7.365
7.347
7.321
7.296
5.915
5.651
3.840
2.816
2.590
2.549
2.337
2.231
2.176
1.588
1.373
1.255
1.108
1.019
0.978
0.879
0.854
0.840
0.000
Integral
0.996
0.289
1.089
1.664
7.409
0.573
0.128
1.793
55.291
8.878
ppm
8
6
4
2
0
Current Data Parameters
NAME 6795 3fcd
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20040602
Time 9 32
INSTRUM DRX300
PROBHD 5 mm Multinu
PULPROG zg
TD 32768
SOLVENT CDCl3
NS 101
DS 0
SWH 6313 131 Hz
FIDRES 0 192661 Hz
AQ 2 5952756 sec
RG 512
DW 79 200 usec
DE 6 00 usec
TE 298 0 K
D1 1 00000000 sec
CHANNEL f1
NUC1 1H
P1 6 88 usec
PL1 -3 00 dB
SFO1 300 1325027 MHz
F2 Processing parameters
SI 16384
SF 300 1299748 MHz
WDW EM
SSB 0
LB 0 10 Hz
GB 0
PC 1 20
1D NMR plot parameters
CX 20 00 cm
F1P 9 819 ppm
F1 2946 91 Hz
F2P -0 456 ppm
F2 -139 85 Hz
PPMCM 0 51424 ppm/cm
HZCM 154 33821 Hz/cm
3FCD
RSIC NO 6795

Fig. 18

doublet of C-5 proton of chromone nucleus is clearly discernible at δ 8.12 (J=7.8 Hz) whereas its counterpart is seen at δ 7.65. The sharp singlet at 8.321 which is usually the diagnostic peak of C-2 proton of chromone moiety appear at the same value as reported in literature[44]. These spectral features, thus, fit better structure **45** which is formed by simple condensation reaction.



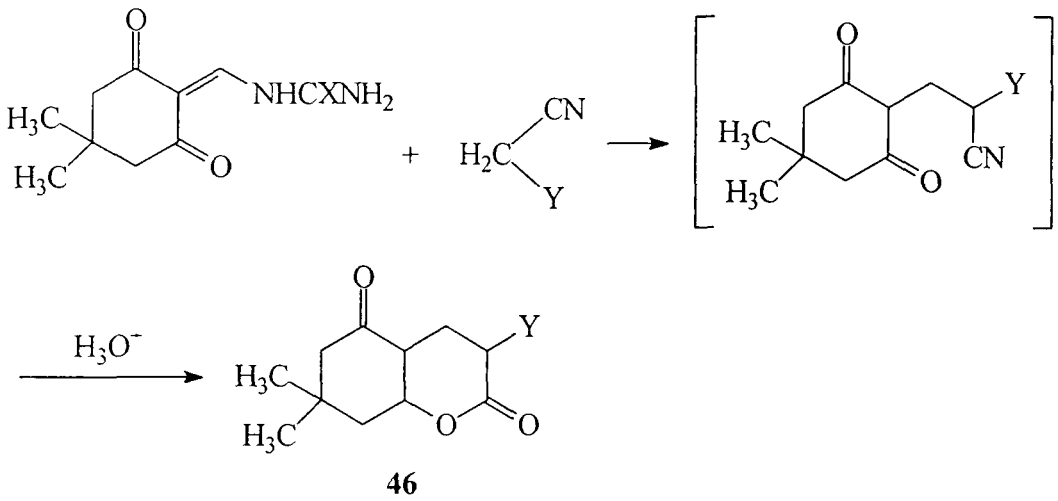
45

The structure **44** is less likely for the compound as the chemical shift for vinylic and under oxygen proton is usually at δ 7.0[45] as against δ 8.32 here. Though the NMR spectrum shows a singlet at δ 5.91 which may be assigned to allylic proton, the integration for this singlet does not match with the integration for other protons. Retro-Diels-Alder cleavage giving fragment ions at m/z 120 and 176 further confirm structure **45** for the compound.

The work on the synthesis of new heterocyclic compounds of biological interest has made extensive use of 4-hydroxycomarin, triacetic acid lactone and their derivatives. This is due to readiness with which these compounds react at position 3 with electrophiles and propensity of 4-position to nucleophilic attack. Another possibility is opening of pyrone ring followed by loss of carbon dioxide or further cyclization. We have discussed here the reaction of enol lactones viz. 4-hydroxycoumarin and triacetic acid lactone with starting materials such as 2-amino-3-formylchromone and 2-ureidomethylenecyclohexane-1,3-dione in order to obtain novel heterocycles.

3.3 The reaction of 4-hydroxycoumarin with 2-ureidomethylene-cyclohexane-1,3-dione:

This reaction was investigated in the context of earlier studies on the reaction of active methylene compounds and 2-ureidomethylene-cyclohexane-1,3-dione which had formed tetrahydrocoumrins[46]. The reaction is, thus, a modified form of Knovenagel reaction for the synthesis of coumarin from salicylaldehyde and malonic ester/malononitrile[47,48]. The authors have chosen here 2-ureidomethylenecyclohexane-1,3-dione in place of salicyldehyde because due to presence of $NHCXNH_2$ (X=O, S) group, 3 position of 2-ureidomethylenecyclohaxane-1,3-dione group acts as an electrophilic centre for neuclophilic attack in the synthesis of 5-oxo-5 ,6,7,8-tetrahydrocoumrins **46** (Scheme 17).

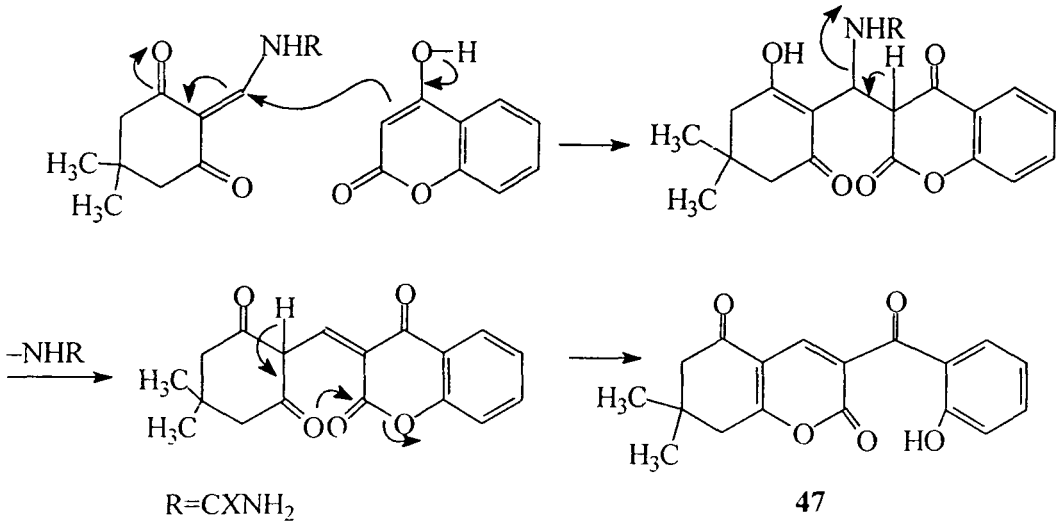


46

Y=$COOCH_3$, $COOC_2H_5$, C_6H_5, COC_6H_5 etc
X=O,S

Scheme 17

It was of interest, therefore, to see what course the reaction would take with 2-ureidomethylenecyclohexane-1,3-dione and 4-hydroxycoumarin. Thus, the compound **47** is expected from the reaction of 2-ureidomethylenecyclohexane-1,3-dione and 4-hydroxycoumarin. The mechanism leading to **47** involves the sequence of reaction shown below



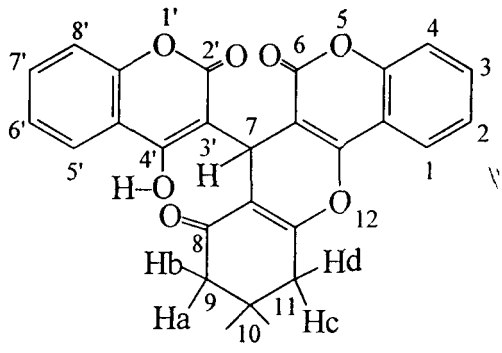
R=$CXNH_2$
X=O,S

47

Scheme 18

It is pertinent to mention here that the reaction of 4-hydroxycoumarin with aldehydes and ketones yields dicoumarols[49].

The reaction however, did not proceed as thought and afforded **48** instead of **47**.



48

The structure **47** was ruled out because it did not give any colour with ferric chloride. Further the mass spectrum (Fig. 19) of the compound which shows M^+ at m/z 456 was also not compatible with structure **47**. The IR spectrum (Fig. 20) for the compound **48** shows a broad band at 3432 cm^{-1} and strong band at 1722 cm^{-1} due to OH and coumarin carbonyl groups. The band at 1616 cm^{-1} accompanied by a shoulder at 1630 cm^{-1}, is due to carbon-carbon double bond and α,β unsaturated carbonyl group. The ^{1}HNMR (Fig. 21) clearly shows two sharp singlets at δ 1.11 and 1.18 for methyl groups. Two methylene groups appear as four doublets at δ 2.16, 2.39 (H_a, H_b, J=15.9 Hz, 15.0 Hz) and 2.64, 2.79 (H_c, H_d, J=17.7 Hz, 17.7 Hz) as a result of gem coupling which takes place among the methylene protons. A sharp

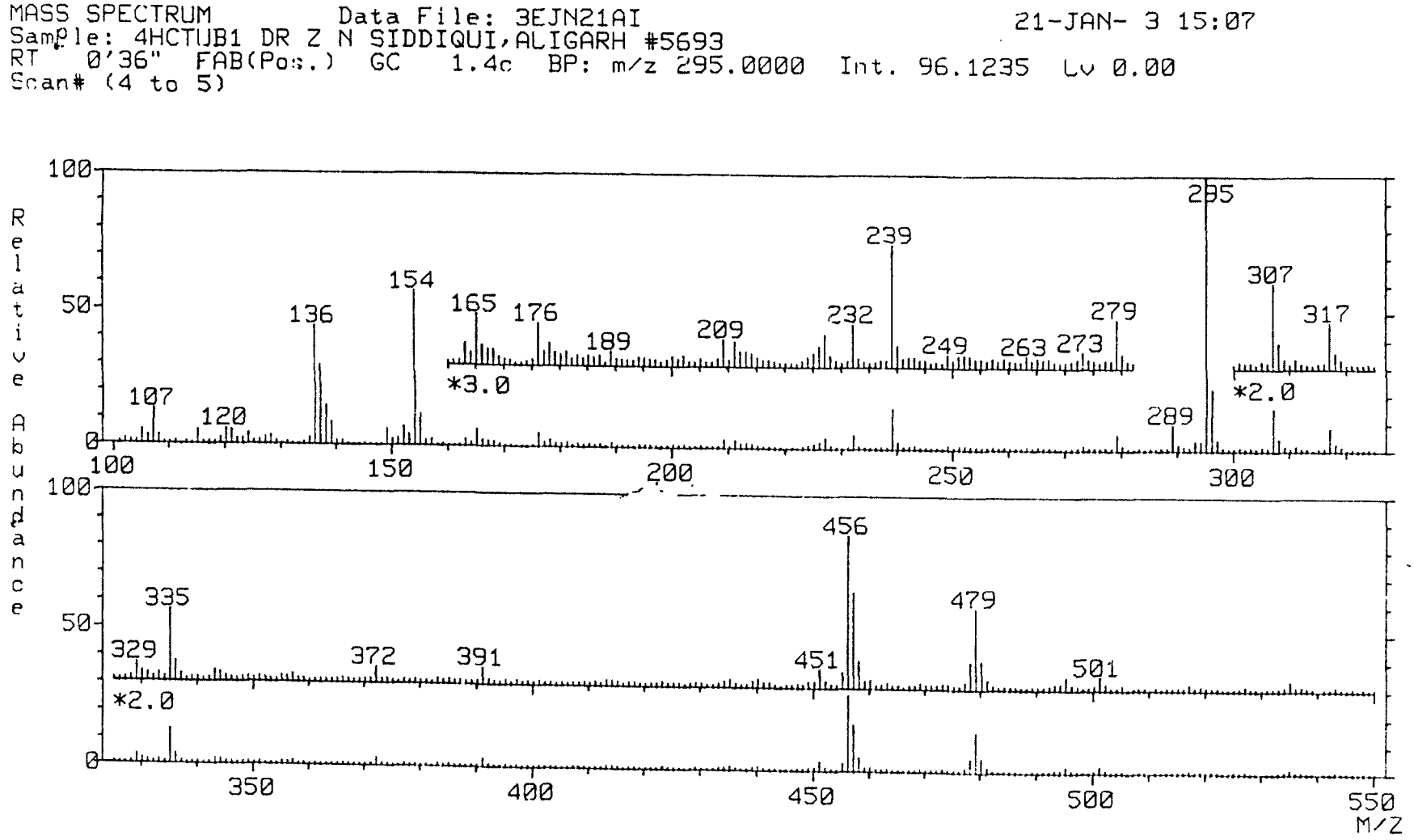

MASS SPECTRUM Data File: 3EJN21AI 21-JAN- 3 15:07
Sample: 4HCTUB1 DR Z N SIDDIQUI,ALIGARH #5693
RT 0'36" FAB(Pos.) GC 1.4c BP: m/z 295.0000 Int. 96.1235 Lv 0.00
Scan# (4 to 5)
Relative Abundance
100
50
0
107
120
136
154
165
176
189
209
232
239
249
263
273
279
289
295
307
317
*3.0
*2.0
100
150
200
250
300
335
329
372
391
451
456
479
501
*2.0
350
400
450
500
550
M/Z

Fig. 19

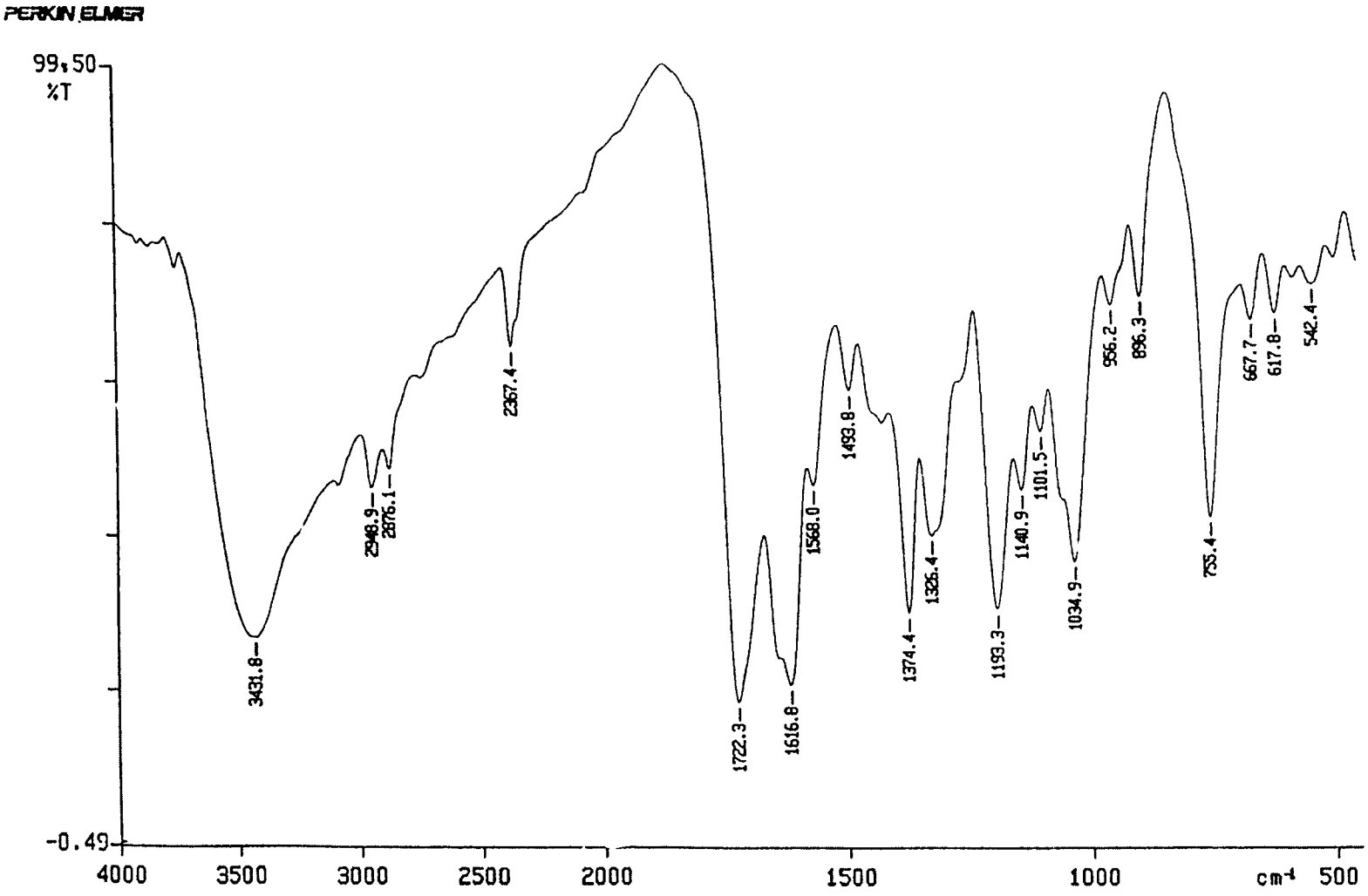

PERKIN ELMER
99.50
%T
-0.49
4000
3500
3000
2500
2000
1500
1000
cm-1
500
3431.8
2946.9
2876.1
2367.4
1722.3
1616.8
1568.0
1493.8
1374.4
1326.4
1193.3
1140.9
1101.5
1034.9
956.2
896.3
755.4
667.7
617.8
542.4

Fig. 20

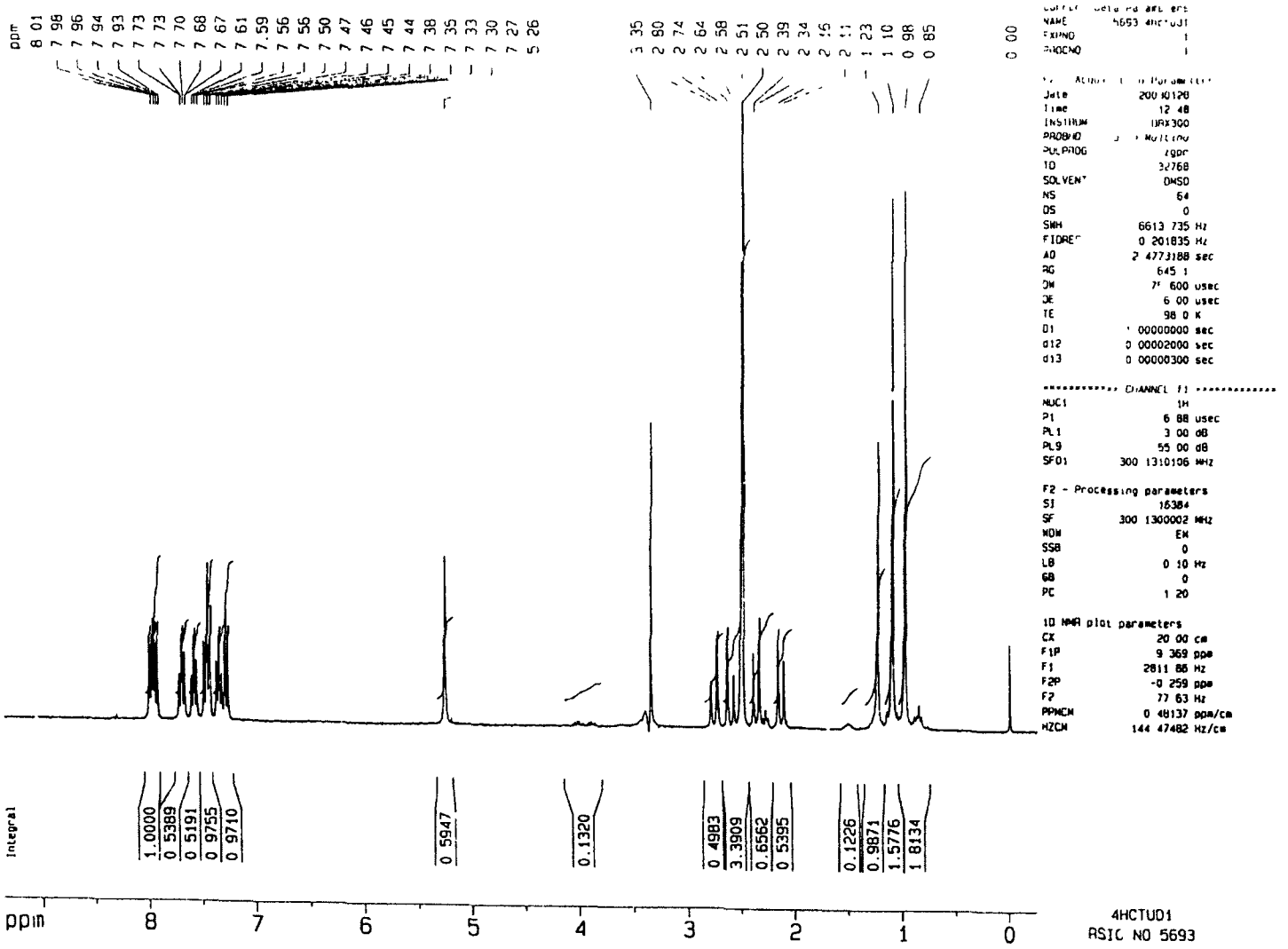
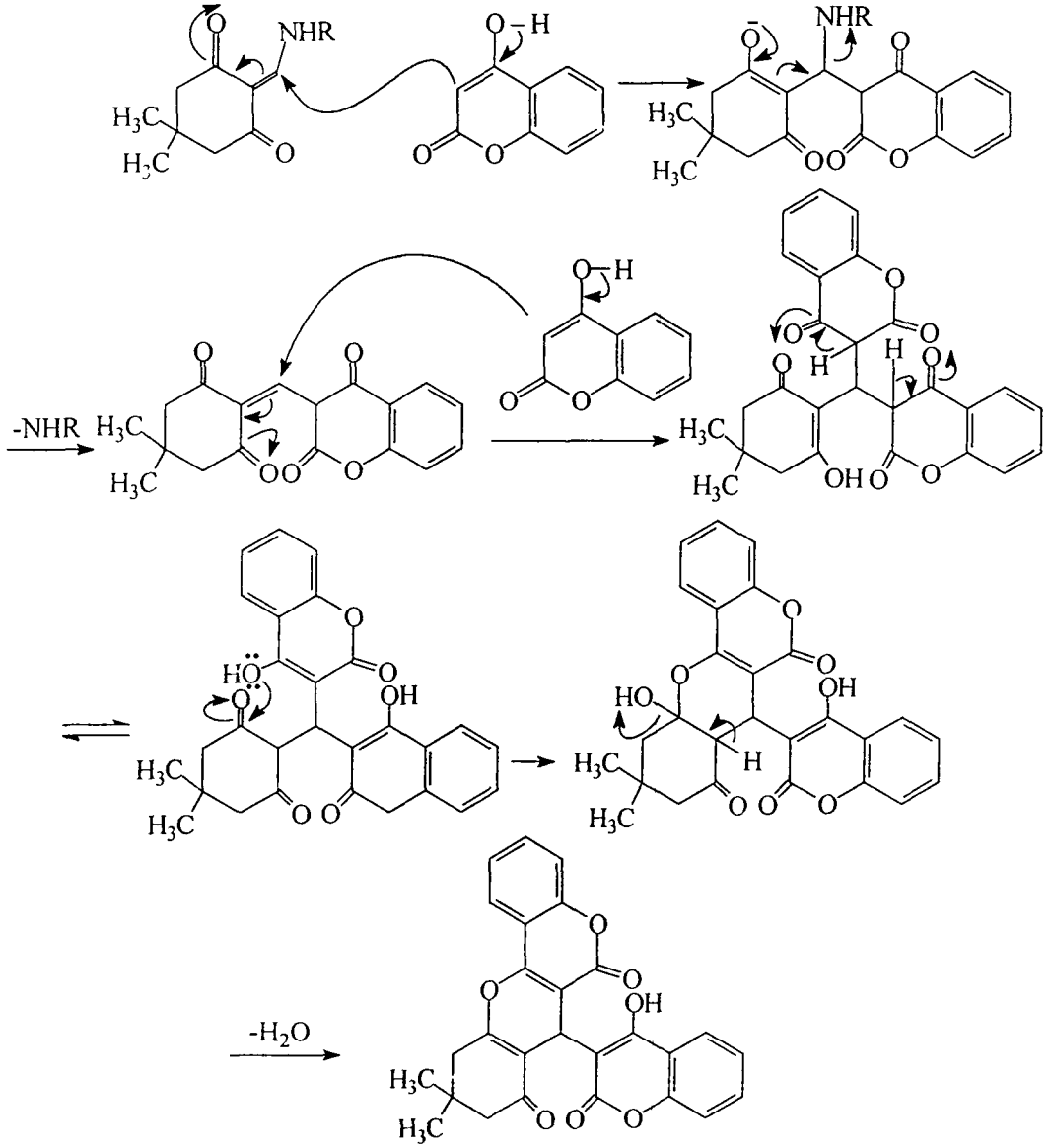

ppm
8 01
7 98
7 96
7 94
7 93
7 73
7 70
7 68
7 67
7 61
7.59
7 56
7 56
7 50
7 47
7 46
7 45
7 44
7 38
7 35
7 33
7 30
7 27
5 26
3 35
2 80
2 74
2 64
2 58
2 51
2 50
2 39
2 34
2 15
2 11
1 23
1 10
0 98
0 85
0 00
NAME 5693 4HCTUD1
EXPNO 1
PROCNO 1
Date 20010120
Time 12 48
INSTRUM DRX300
PROBHD
PULPROG zgpr
TD 32768
SOLVENT DMSO
NS 64
DS 0
SWH 6613 735 Hz
FIDRES 0 201835 Hz
AQ 2 4773188 sec
RG 645 1
DW 75 600 usec
DE 6 00 usec
TE 98 0 K
D1 1 00000000 sec
d12 0 00002000 sec
d13 0 00000300 sec
CHANNEL f1
NUC1 1H
P1 6 88 usec
PL1 3 00 dB
PL9 55 00 dB
SF01 300 1310106 MHz
F2 - Processing parameters
SI 16384
SF 300 1300002 MHz
WDW EM
SSB 0
LB 0 10 Hz
GB 0
PC 1 20
1D NMR plot parameters
CX 20 00 cm
F1P 9 369 ppm
F1 2811 86 Hz
F2P -0 259 ppm
F2 77 63 Hz
PPMCM 0 48137 ppm/cm
HZCM 144 47482 Hz/cm
Integral
1.0000
0 5389
0 5191
0 9755
0 9710
0 5947
0 1320
0 4983
3 3909
0 6562
0 5395
0 1226
0 9871
1 5776
1 8134
ppm
8
7
6
5
4
3
2
1
0
4HCTUD1
RSIC NO 5693

Fig. 21

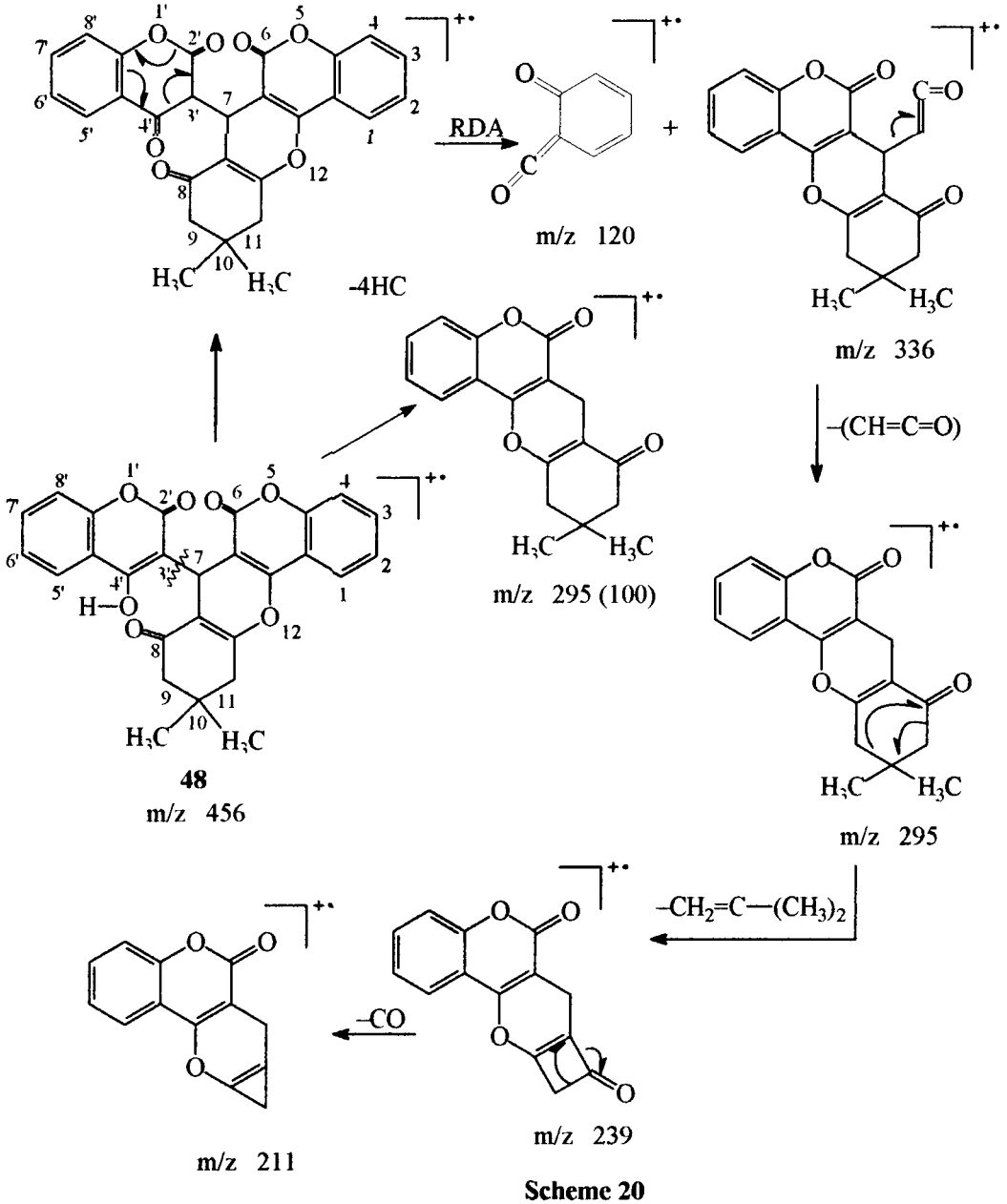
singlet integrating for one proton at δ 5.26 has been assigned to methine (H-7) proton. The aromatic region clearly evidences the presence of two coumarin moieties in the form of two ortho coupled doublets of H-5' and H-1 protons at δ 8.0 and 7.9. The remaining six protons of coumarin nuclei appear as multiplet situated at δ 7.73-7.27. This suggests that in the formation of product **48** two 4-hydroxycoumarin molecules are involved as shown below:



48

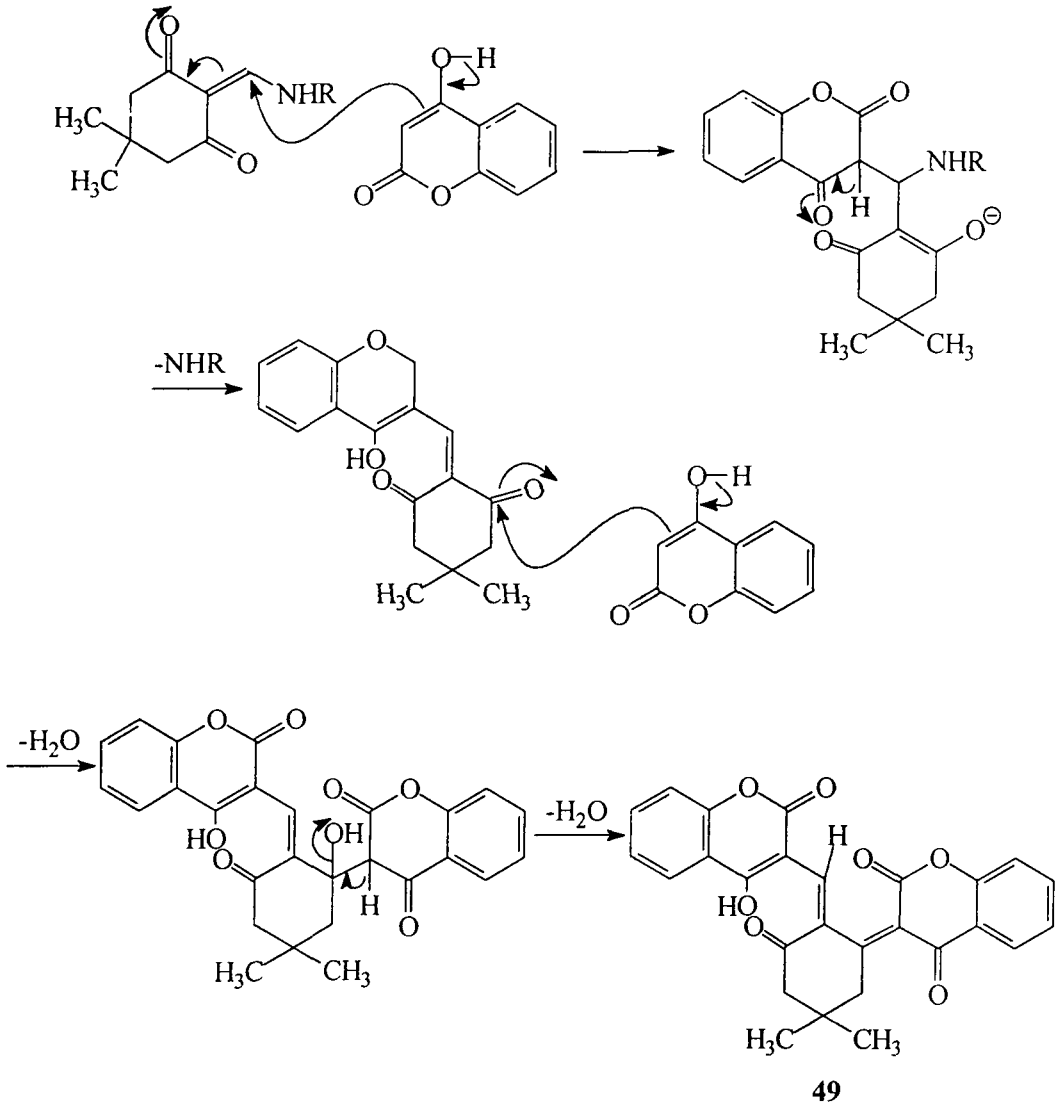
Scheme 19

The compound **48** is further confirmed by its mass spectrum (Fig 19) showing M^+ at 456 The base peak at m/z 295 is obtained by loss of 4-hydroxycoumarin moiety from molecular ion peak through rout A or B involving R D A cleavage (Scheme 20)



Scheme 20

It has to be noted here that these spectral features can fit also structure **49**, which can be rationalized on the basis of the mechanism shown in Scheme 21.



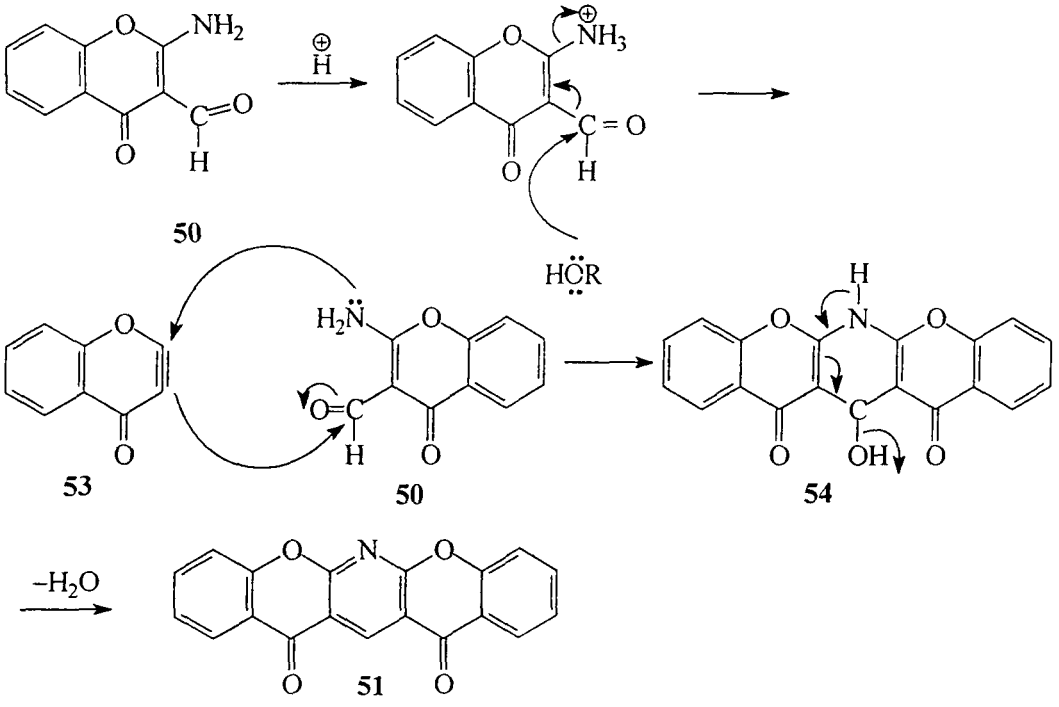
49

Scheme 21

This structure is, however, less probable as the methene proton usually appears as a singlet in the region δ 8.2-8.4[50] as against δ 5.26 here.

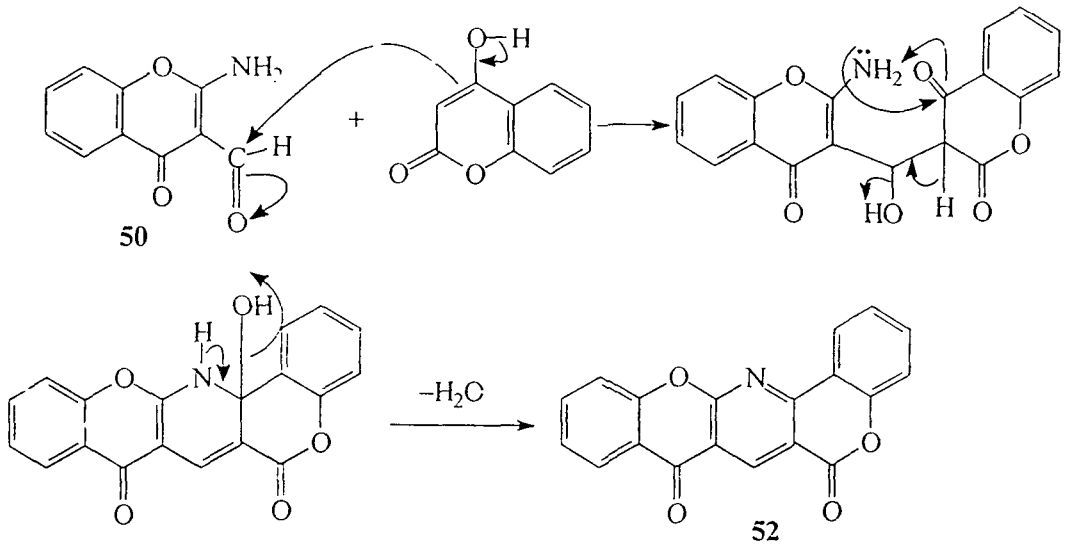
3.4 The reaction of 2-amino-3-formylchromone with triacetic acid lactone:

2-Amino-3-formylchromone **50** is obtained easily by treatment of 3-formylchromone with hydroxylammonium sulfate followed by ring opening and cyclization under basic conditions[51]. Schurreit Thomas has synthesised pentacycles **51** and **52** from **50** and 4-hydroxycoumarin under strong acidic conditions[52] (Scheme 22, 23). The mechanism leading to product **51** involves formation of intermediate **53** similar to benzyne by elimination of formyl group and ammonia from **50**. The intermediate **53** then adds to another molecule of **50** followed by dehydration of the adduct **54** to form **51**.



Scheme 22

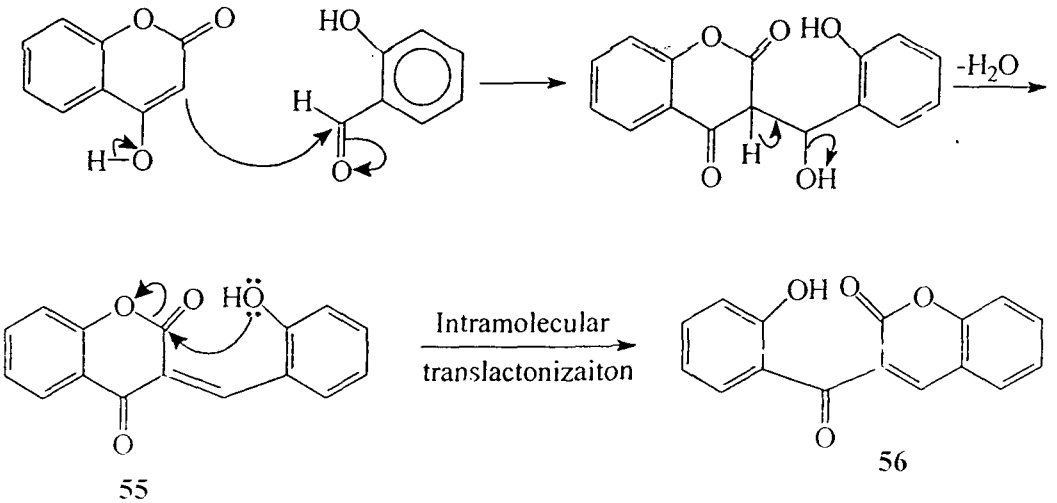
The product **52**, however, is obtained by simple condensation of **50** and 4-hydroxycoumarin.



Scheme 23

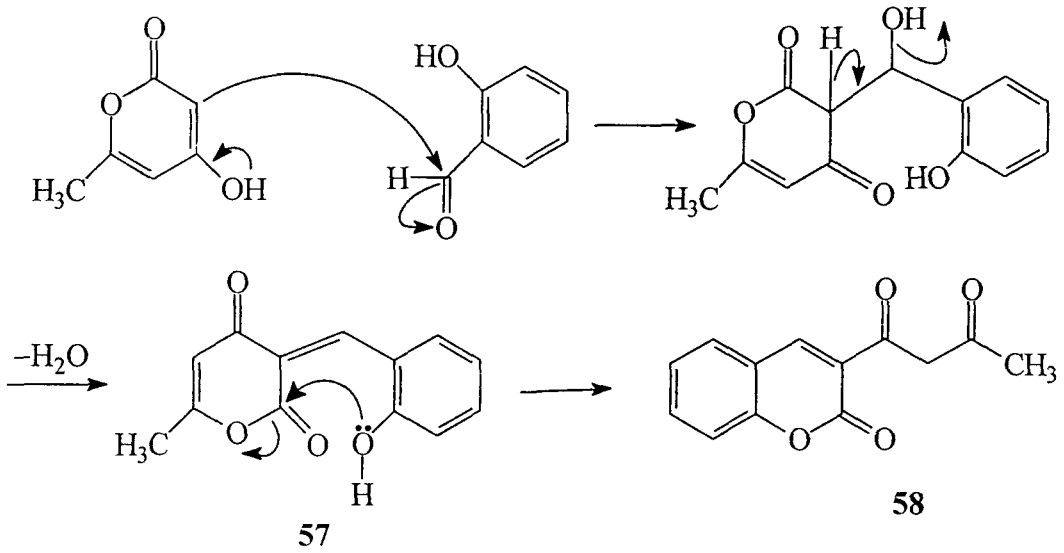
Since triacetic acid lactone (TAL) shows nucleophilic properties similar to 4-hydroxycoumarin due to presence of double bond at position 4, attempt was made to synthesised novel polyketo compounds employing **50** as starting material.

Enol lactones react with salicyldehyde to give a product who structure was assigned as **55** by earlier worker[53]. The reaction was further investigated because such structures were found to be unstable and therefore, structure **55** was revised to **56**[54].



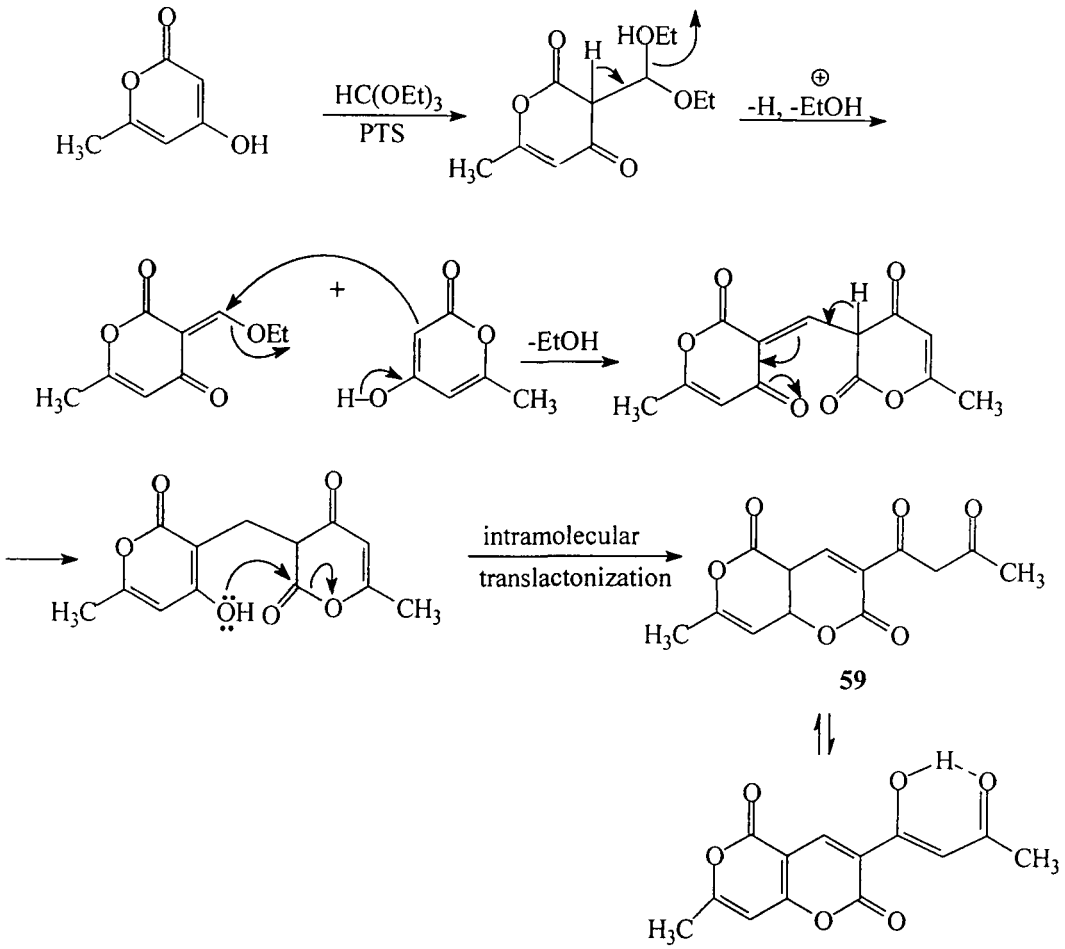
Scheme 24

In a series of papers Spanish workers[55] have discussed at length that structures similar to **55**, generally rearrange to **56** through intramolecular translactonization. Thus, TAL when reacts with salicyldehyde, the intermediate **57** rapidly undergoes intramolecular translactonization to give **58** (Scheme 25).



Scheme 25

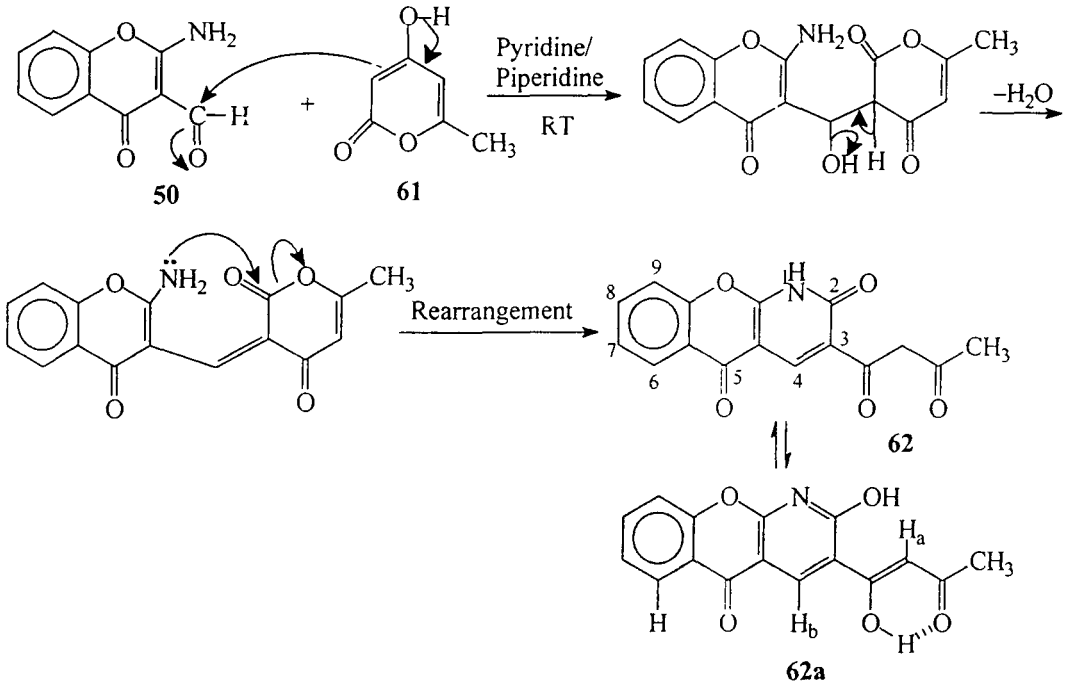
In an attempt to synthesize 3-formyl derivative of TAL which, was needed in the context of another project with triethylorthoformate, the reaction mixture, however, gave the rearranged product **59**[56] (Scheme 26).



Scheme 26

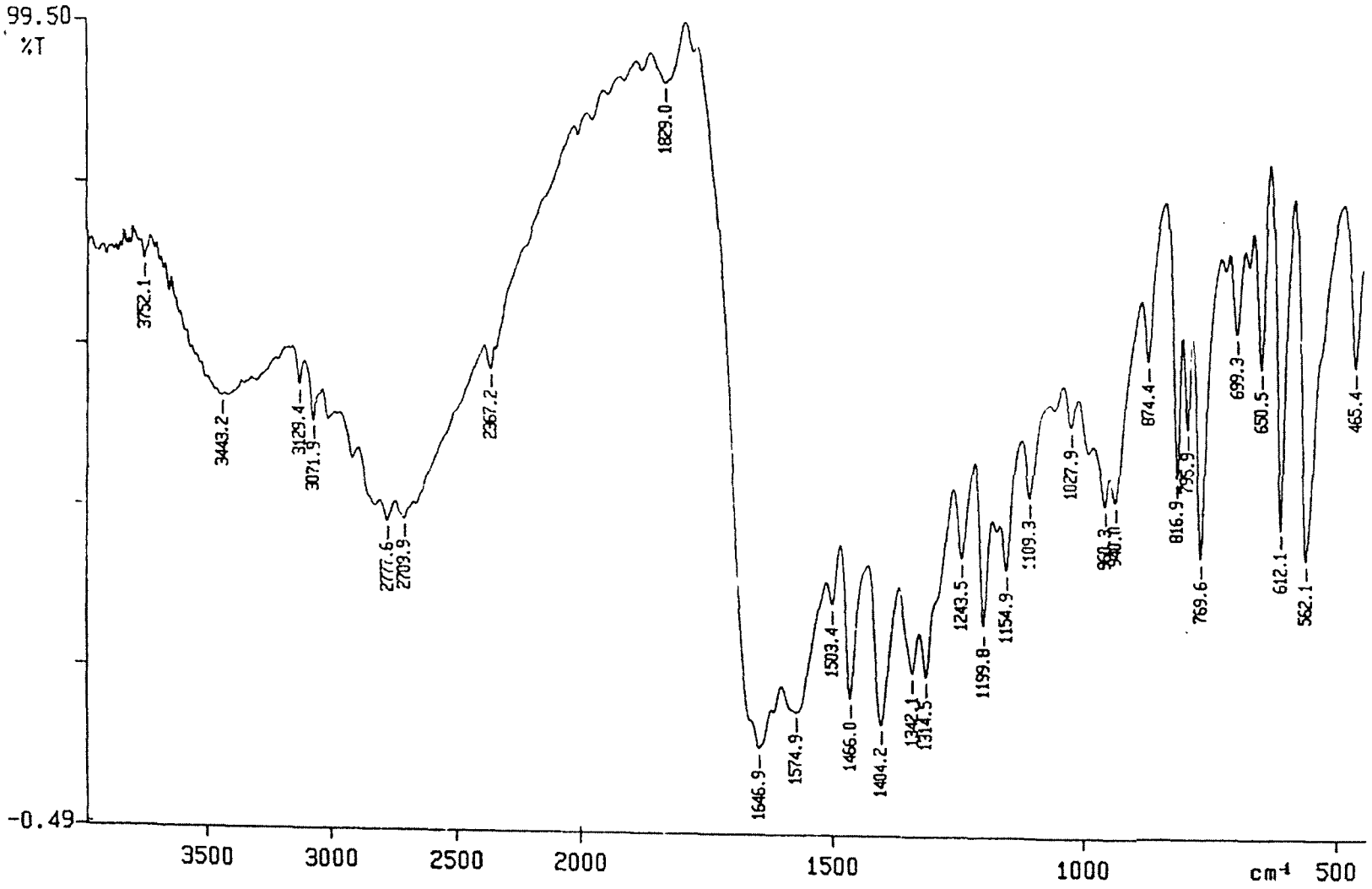
These studies reveal that the intramolecular translactonization takes place by opening up of the lactone ring by nucleophilic altack of hydroxyl group of salicylaldehyde. Since the amino group shows better nucleophilic property than hydroxyl group, it was thought that the intramolecular translactonization will occur much easily if OH group is substituted by NH_2 group in a compound to afford the rearranged product. **50** was, therefore, selected for carrying out such types of rearrangement reactions as it contained both NH_2 and CHO chromophores at positions similar to OH and CHO groups in salicylaldehyde. The reaction afforded **62** as felt by conducting the reaction of 2-amino-3-formylchromone **50** with TAL **61.** A

positive ferric chloride test and strong yellow colour of the product due to extended conjugation further supported that this actually has happened (Scheme 27). The ease with which reaction proceeds is indicated by the fact that product **62** is formed at room temperature and in quantitative yield.



Scheme 27

The IR spectrum (Fig. 22) showed strong bands at 1647 with shoulders at 1652 and 1642 cm^{-1} for chromone, lactam and chelated carbonyl groups. In the ^1H nmr spectrum (Fig. 23) methyl singlet was clearly discernible at δ 2.28. A sharp singlet at δ 7.09 was assigned to H_a proton. The presence of chromone ring was established by ortho coupled doublet of C_6 proton at δ 8.2 (J=9 Hz). The most deshielded olefinic proton in the compound H_b appeared as a sharp singlet at δ 8.86. Two broad singlets which appeared at δ 16.5 and 13.5 (D_2O exchangeable) indicated the presence of two OH groups in the compound. Thus, **62,** also existed in


99.50
%T
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3752.1
3443.2
3129.4
3071.9
2777.6
2709.9
2367.2
1829.0
1646.9
1574.9
1503.4
1466.0
1404.2
1342.1
1314.5
1243.5
1199.8
1154.9
1109.3
1027.9
960.3
940.1
874.4
816.9
795.9
769.6
699.3
650.5
612.1
562.1
465.4
3500
3000
2500
2000
1500
1000
cm-1
500

Fig. 22

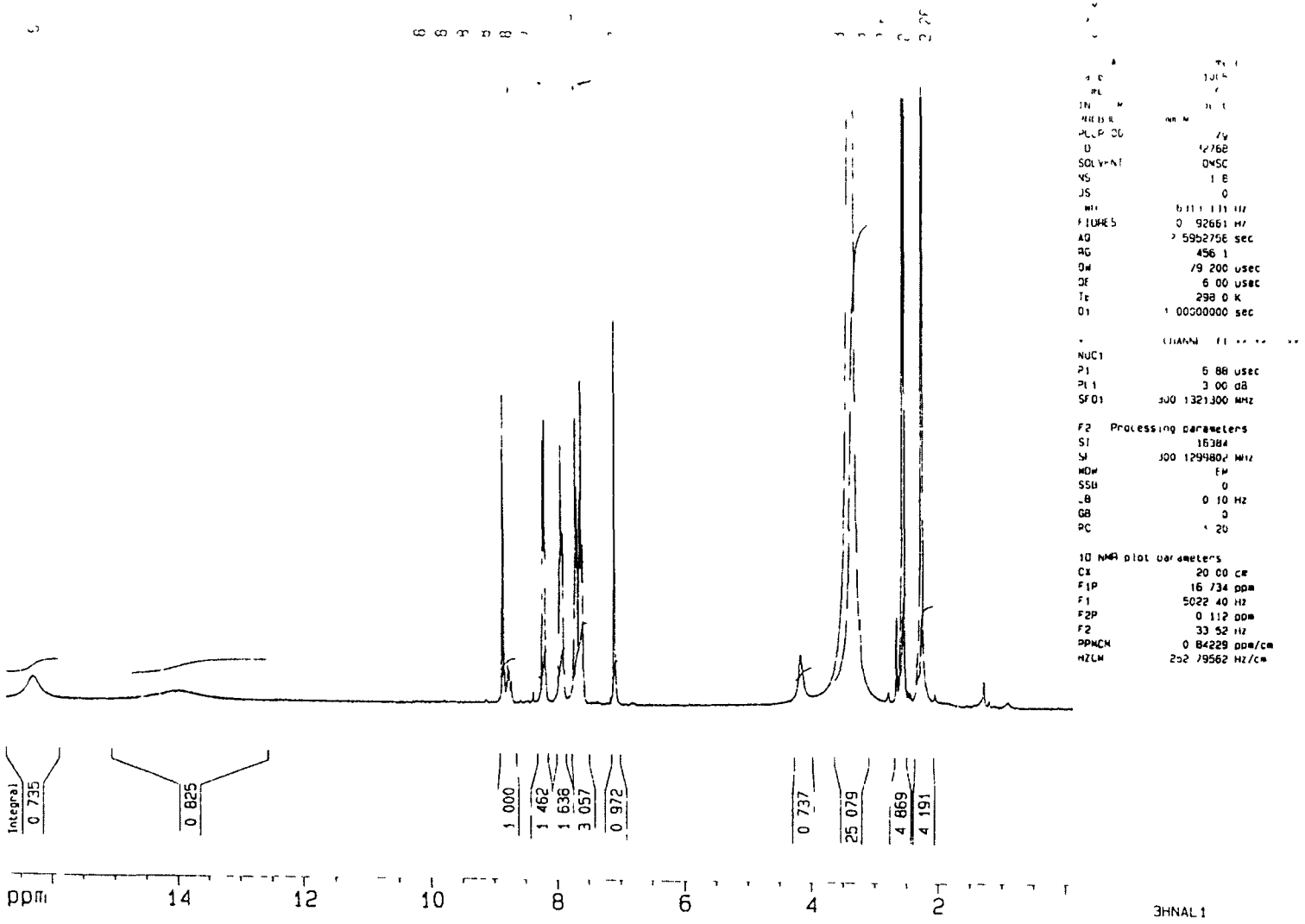

Integral
0 735
0 825
1 000
1 462
1 636
3 057
0 972
0 737
25 079
4 869
4 191
ppm
14
12
10
8
6
4
2
3HNAL1
NUC1
P1 6 88 usec
PL1 3 00 dB
SFO1 500 1321300 MHz
F2 Processing parameters
SI 16384
SW 500 1299802 MHz
WDW EM
SSB 0
LB 0 10 Hz
GB 0
PC 1 20
1D NMR plot parameters
CX 20 00 cm
F1P 16 734 ppm
F1 5022 40 Hz
F2P 0 112 ppm
F2 33 52 Hz
PPMCM 0 84229 ppm/cm
HZCM 252 79562 Hz/cm

Fig. 23

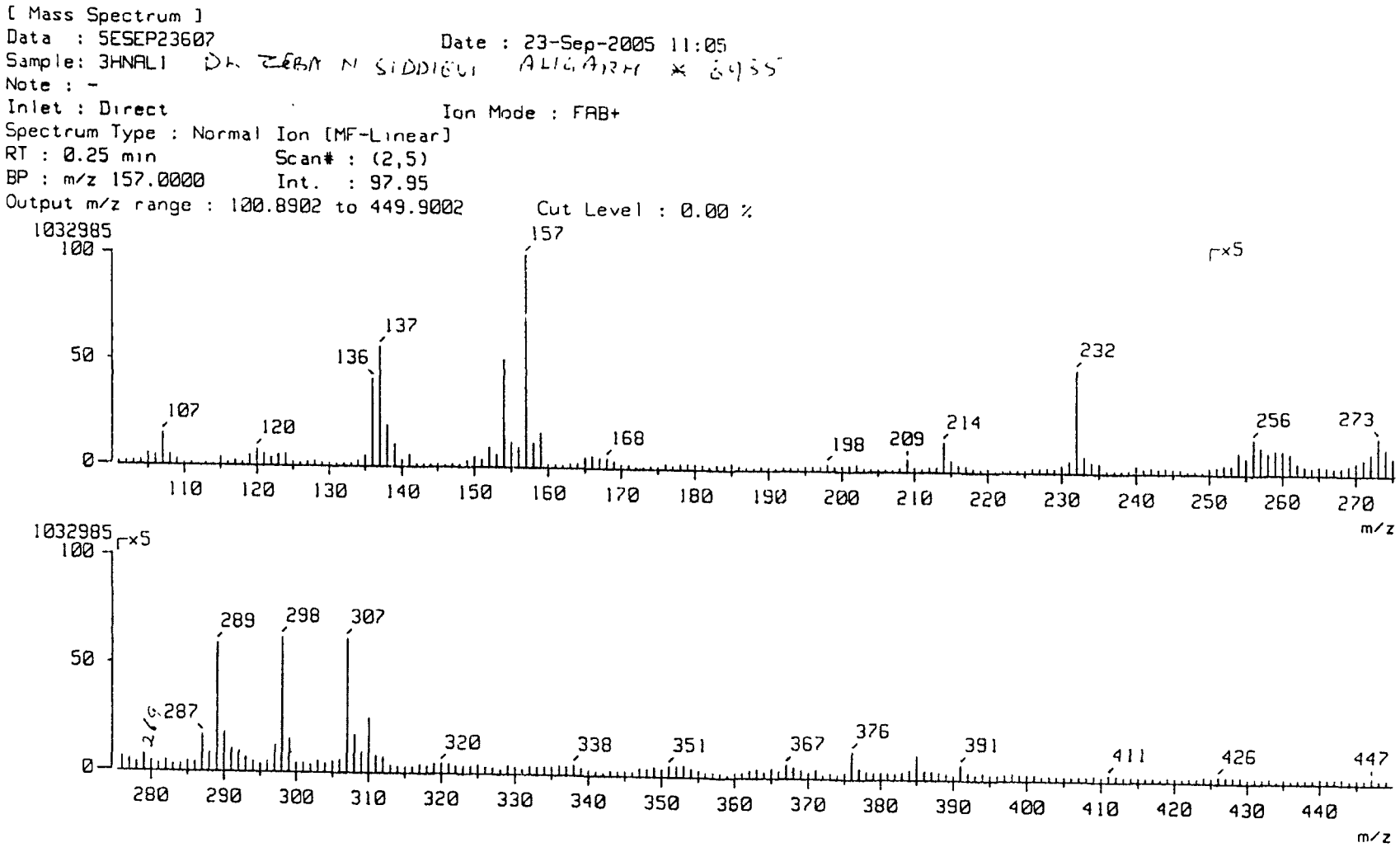
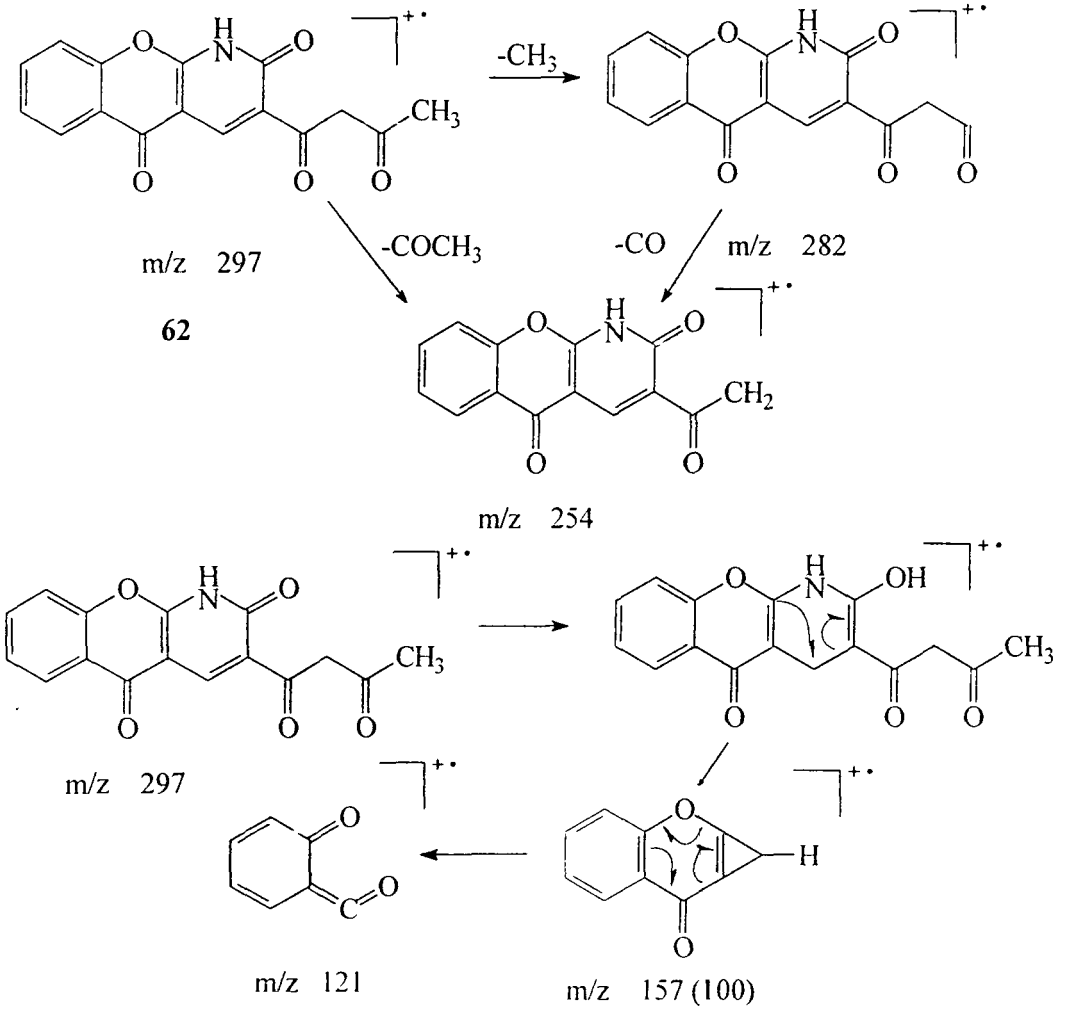
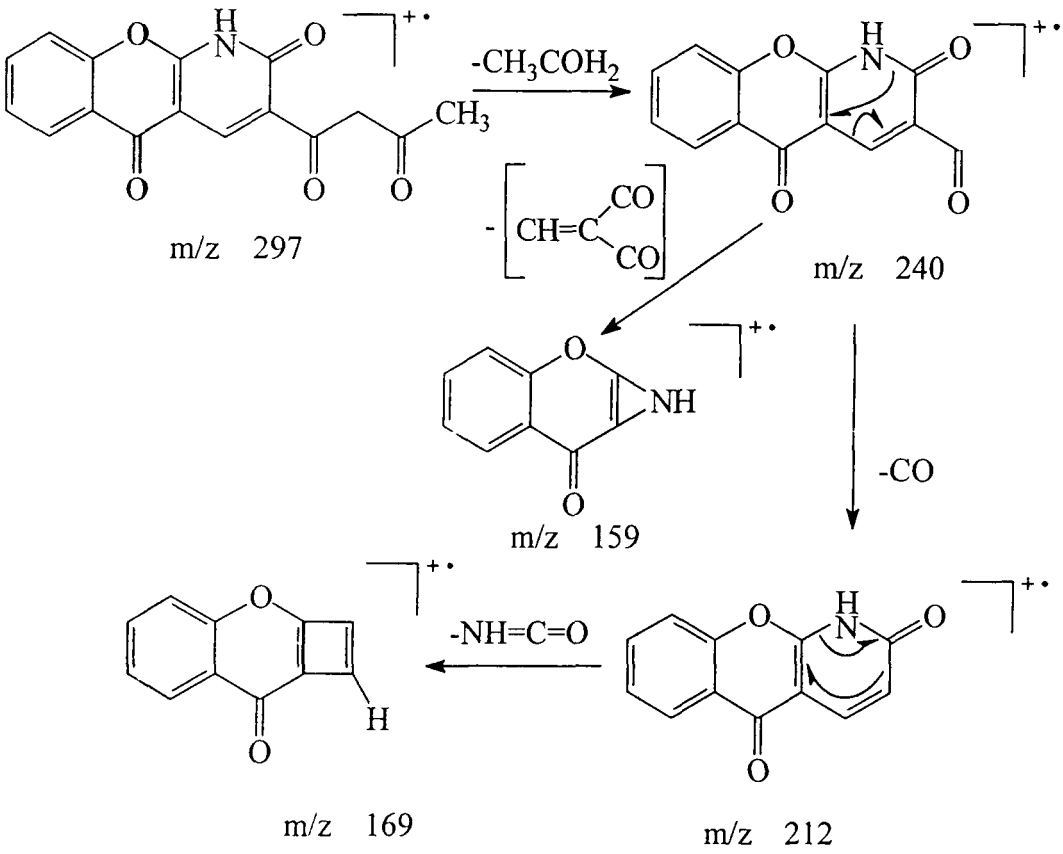

[Mass Spectrum]
Data : 5ESEP23607
Date : 23-Sep-2005 11:05
Sample: 3HNAL1 DR. ZEBA N SIDDIQUI ALIGARH × 6455
Note : -
Inlet : Direct
Ion Mode : FAB+
Spectrum Type : Normal Ion [MF-Linear]
RT : 0.25 min
Scan# : (2,5)
BP : m/z 157.0000
Int. : 97.95
Output m/z range : 100.8902 to 449.9002
Cut Level : 0.00 %
1032985
157
×5
137
136
232
107
120
214
256
273
168
198
209
m/z
1032985
×5
289
298
307
287
320
338
351
367
376
391
411
426
447
m/z

Fig. 24

its tautomeric form **62a.** The structure **62** for the compound was supported by its mass spectrum (Fig. 24) which showed M^+ at m/z 297. The smaller peaks at m/z 282 and 254 indicated losses of CH_3 and CH_3CO groups. A loss of 57 mass units corresponding to elimination of CH_3COCH_2 group gave fragment ion at m/z 240. The mass spectrum of 3-acetoacetylcoumarin studied by Dean et al[57] is also characterized by loses of CH_3CO and CH_3COCH_2 groups. However, in our case the base peak is obtained as a result of loss of $CH{=}C(CO)_2$ grouping from fragment ion m/z 240. The other relevant peaks are obtained as shown in Scheme 28.

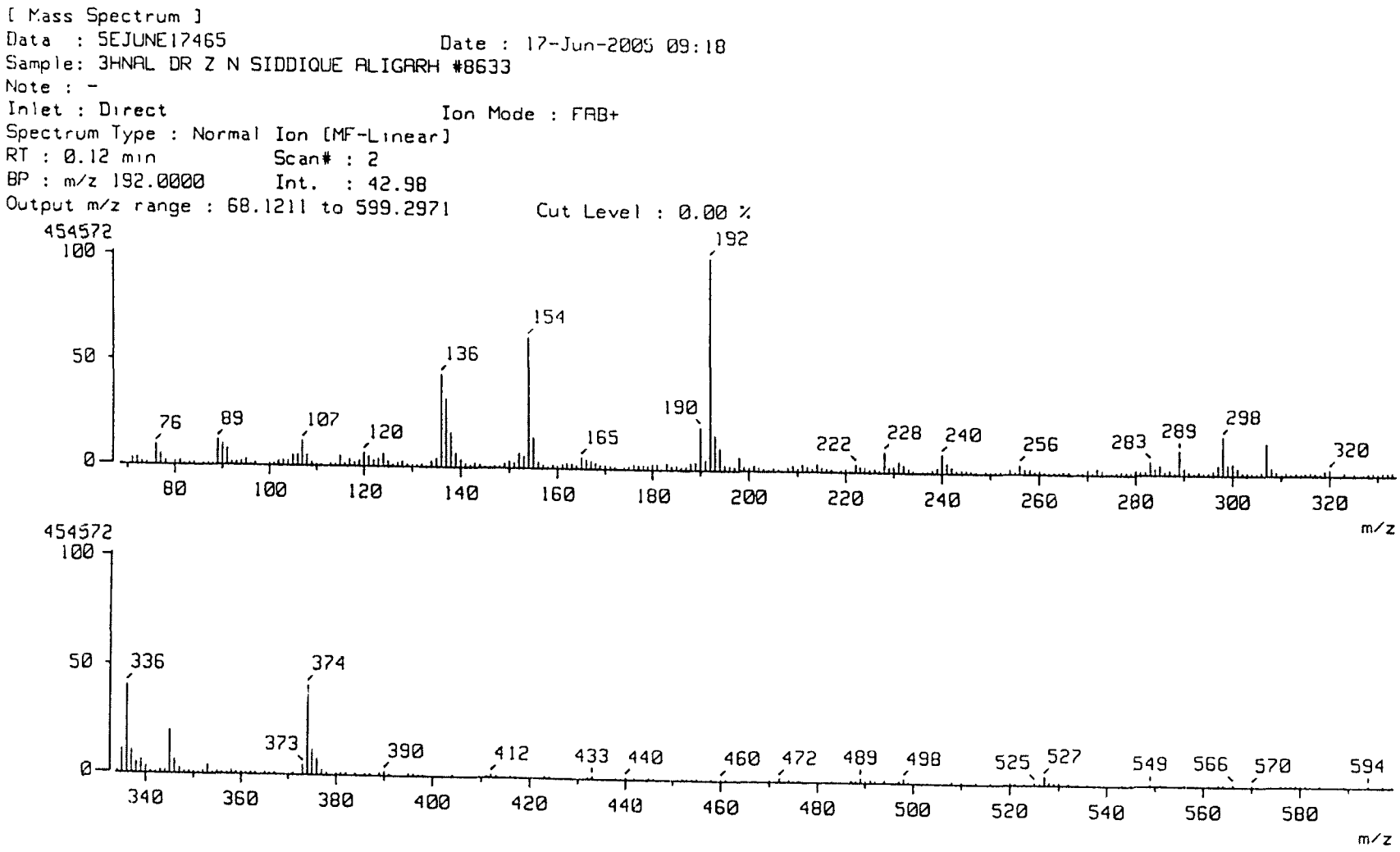
Transformation of **62** to other heterocyclic compounds is in progress.





Scheme 28

While attempting the synthesis of 3-acetoacetyl pyranopyridone, **62,** lebelled as 3HNaL-1 under different reaction conditions, a light yellow product was isolated by conducting the reaction of 2-amino-3-formylchromone **50** with triacetic acid lactone **61** under reflux in the presence of potassium acetate. It was lebelled as 3HNaL and gave light brownish blue colour with ferric chloride. The intensity of the colour was somewhat less than 3HNaL-1, **62**. The compound, analyzed for $C_{22}H_{15}O_5N$, showed odd molecular ion peak at m/z 373 in the mass spectrum (Fig. 25). Since the molecular weight of 2-amino-3-formylchromone and triacetic acid lactone are 189 and 126 mass units, the addition product would have the molecular weight 189+126=315 which is less than the required molecular weight for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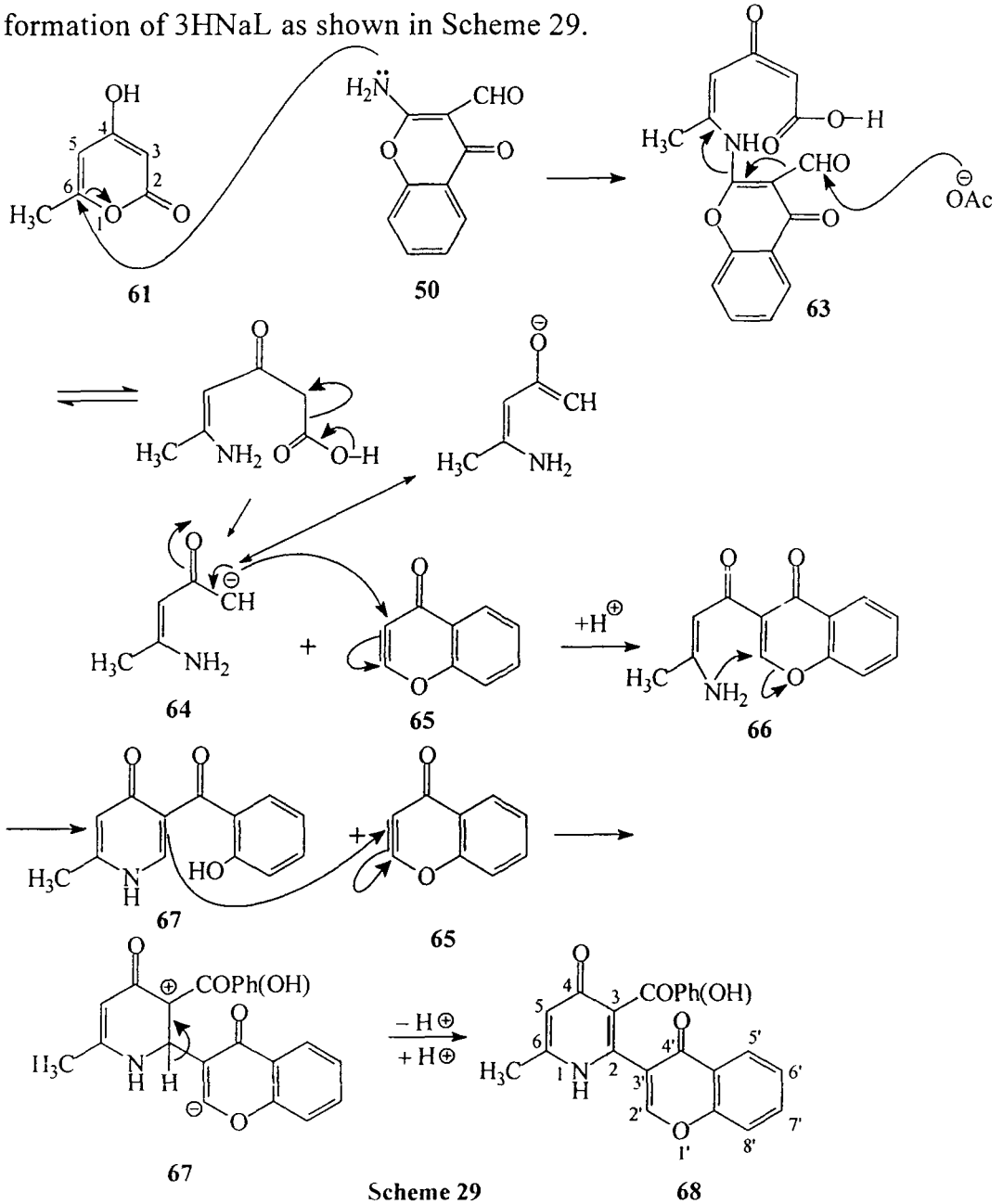
Sample: 3HNAL DR Z N SIDDIQUE ALIGARH #8633
Note : -
Inlet : Direct Ion Mode : FAB+
Spectrum Type : Normal Ion [MF-Linear]
RT : 0.12 min Scan# : 2
BP : m/z 192.0000 Int. : 42.98
Output m/z range : 68.1211 to 599.2971 Cut Level : 0.00 %
454572
100
50
0
192
154
136
190
76
89
107
120
165
222
228
240
256
283
289
298
320
80
100
120
140
160
180
200
220
240
260
280
300
320
m/z
454572
100
50
0
336
374
373
390
412
433
440
460
472
489
498
525
527
549
566
570
594
340
360
380
400
420
440
460
480
500
520
540
560
580
m/z

Fig. 25

the compound. Since the NMR spectrum (Fig. 26) shows presence only one methyl singlet and 8 aromatic protons, it is obvious that the formation of 3HNaL with molecular weight 373, would require incorporation of two units of 2-amino-3-formylchromone **50** and one unit of triacetic acid lactone **62** (2 x 189+126=504). This is followed by loss of fragments having 131 mass units (504-373=131). The reaction, thus, involves addition-elimination type of reaction in the formation of 3HNaL. One has, therefore, to look for a sequence of reactions which can cleave the starting materials in the formation of 3HNaL as shown in Scheme 29.



Scheme 29

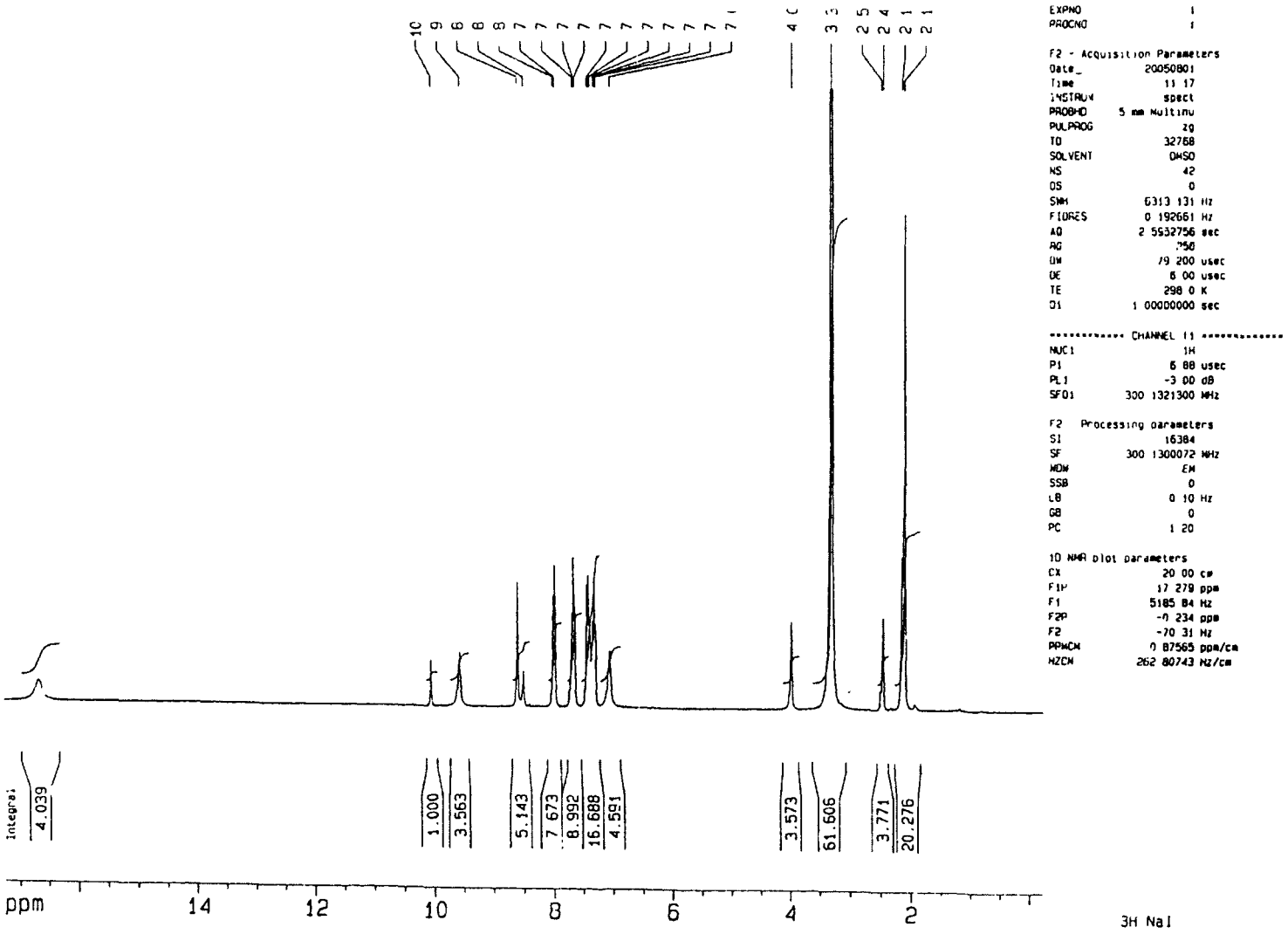
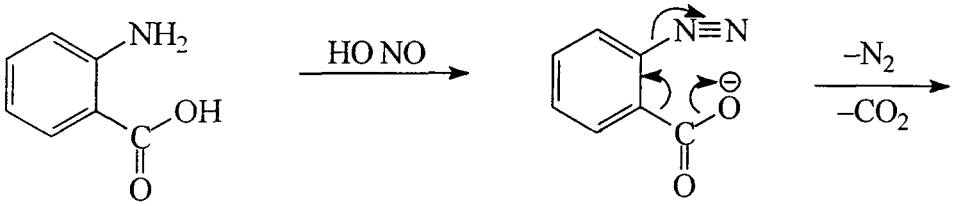
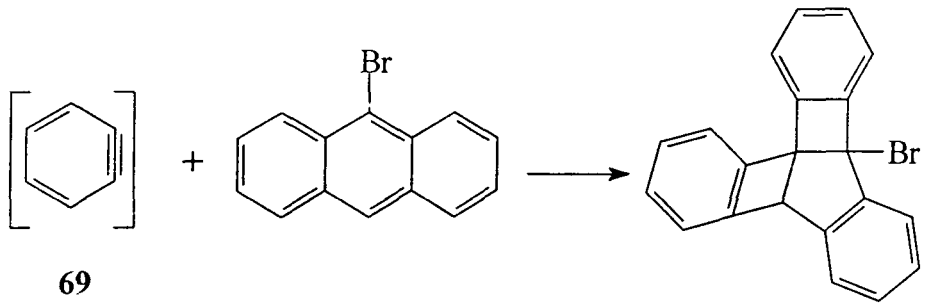

EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20050801
Time 11 17
INSTRUM spect
PROBHD 5 mm Multinu
PULPROG zg
TD 32768
SOLVENT DMSO
NS 42
DS 0
SWH 6313 131 Hz
FIDRES 0 192661 Hz
AQ 2 5532756 sec
RG 750
DW 79 200 usec
DE 6 00 usec
TE 298 0 K
D1 1 00000000 sec
CHANNEL f1
NUC1 1H
P1 6 88 usec
PL1 -3 00 dB
SFO1 300 1321300 MHz
F2 Processing parameters
SI 16384
SF 300 1300072 MHz
WDW EM
SSB 0
LB 0 10 Hz
GB 0
PC 1 20
1D NMR plot parameters
CX 20 00 cm
F1P 17 279 ppm
F1 5185 84 Hz
F2P -0 234 ppm
F2 -70 31 Hz
PPMCM 0 87565 ppm/cm
HZCM 262 80743 Hz/cm
integral
4.039
1.000
3.563
5.143
7.673
8.992
16.688
4.591
3.573
61.606
3.771
20.276
ppm
14
12
10
8
6
4
2
3H NaI

Fig. 26

Assuming that amino group of **50** attacks on C-6 carbon of triacetic acid lactone **61**. the initial product would be **63** which undergoes decarboxylation and elimination reaction removing CHO and NH_2 groups to form the carbanion **64** and intermediate **65.** The addition of carbanion **64** to **65** takes place to give **66**. This undergoes cyclization and ring opening of pyrone ring to give **67**. The double bond of pyridone **67** reacts further with another molecule of **65** to give the final product **68**.

The formation of intermediate **65** is similar to that observed in the synthesis of 9-bromotriptycene **70** from anthranilic acid and 9-bromo naphthalene involving the intermediate benzyne **69** through elimination of N_2 and CO_2[58].

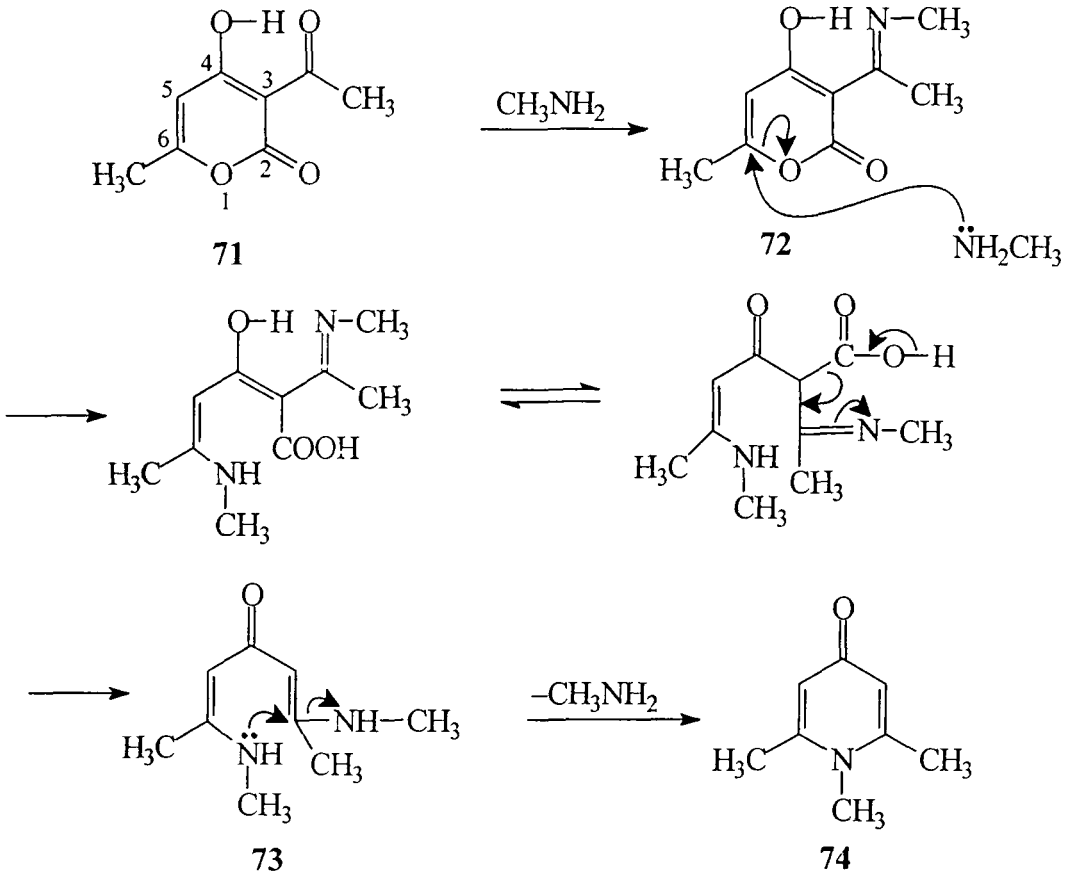




69 **70**

The sequence of reactions (Scheme 29) leading to the formation of product **68** is supported by the work of S. Garrat. Her work is about the reaction of dehydroacetic acid **71** and alkyl amines[59]. According to her results, dehydroacetic acid reacts with methylamine to form the

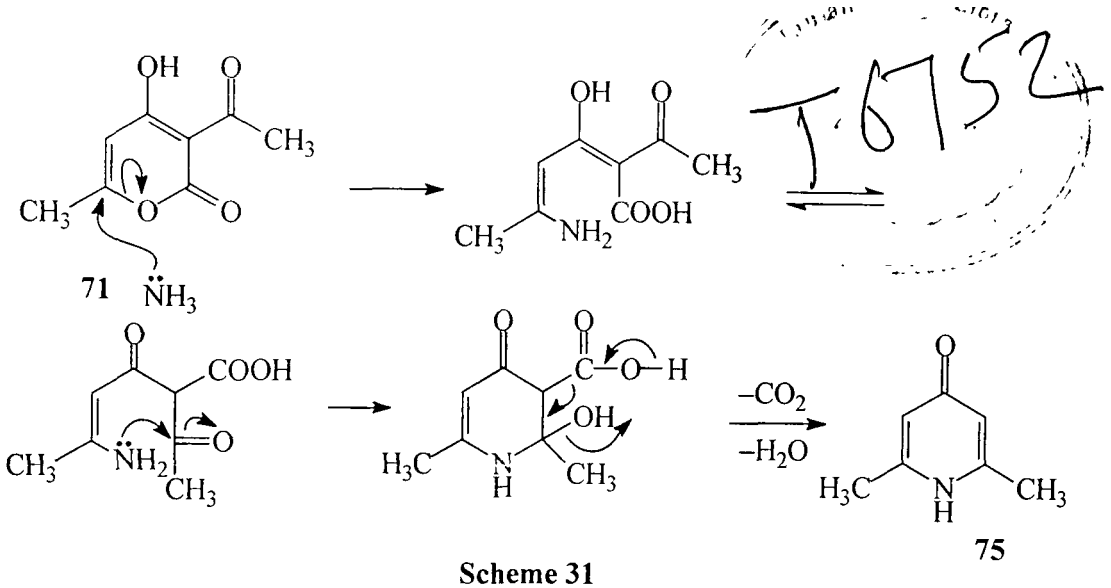
Schiff's base **72**. On further reaction with methylamine, **72** is converted into bis-2,7-methylaminohepta-2, 5-dien-4-one **73**. The final product **74** is formed by removal of one molecule of methylamine from **73** (Scheme 30). The reaction, thus, involves attack by methylamine on C-6 carbon of dehydroacetic acid **71** in the formation of lutidone, **74**.



71 72 73 74

Scheme 30

The attack of amino group an C-6 carbon of dehydroacetic acid is further supported by the work of Japanese workers[60]. In their findings, regarding the reaction of dehydroacetic acid with ammonia, the pyridone, **75** is formed by the attack of nucleophile on C-6 carbon. (Scheme 31)



Scheme 31

These studies are relevant in the present context to the extent that they show the possibility of nucleophilic attack at C-6 carbon in preference to attack at the lactone carbonyl. Drawing on the analogy of dehydroacetic acid it is, thus, possible that amino group of 2-amino-3-formylchromone, **50** attacks on C-6 carbon of triacetic acid lactone **61** to form the product, **68.**

The IR spectrum of **68** (Fig. 27) shows a strong band at 1616 with shoulders at 1650 and 1600 in addition to bands for NH and OH groups at 3249 and 3398 cm^{-1} respectively. The strong absorption band at 1616 cm^{-1} may be assigned to pyridone carbonyl group as the value for pyridones have been reported is the range 1500-1700 cm^{-1} including pyrones and pyrimidones[61]. The shoulders at 1650 and 1600 cm^{-1} have been assigned to chromone carbonyl and o-hydroxybenzoyl groups. The NMR spectrum (Fig. 26) clearly shows a sharp singlet for methyl group at δ 2.13 with a slight notch which may be due to long range coupling of methyl hydrogens with C-5 proton. This value is, however, slightly higher as the chemical shift of C-5 proton in compounds derived from triacetic acid lactone is usually δ 6.5-6.7[62] as against δ 7.07 here. The singlet at

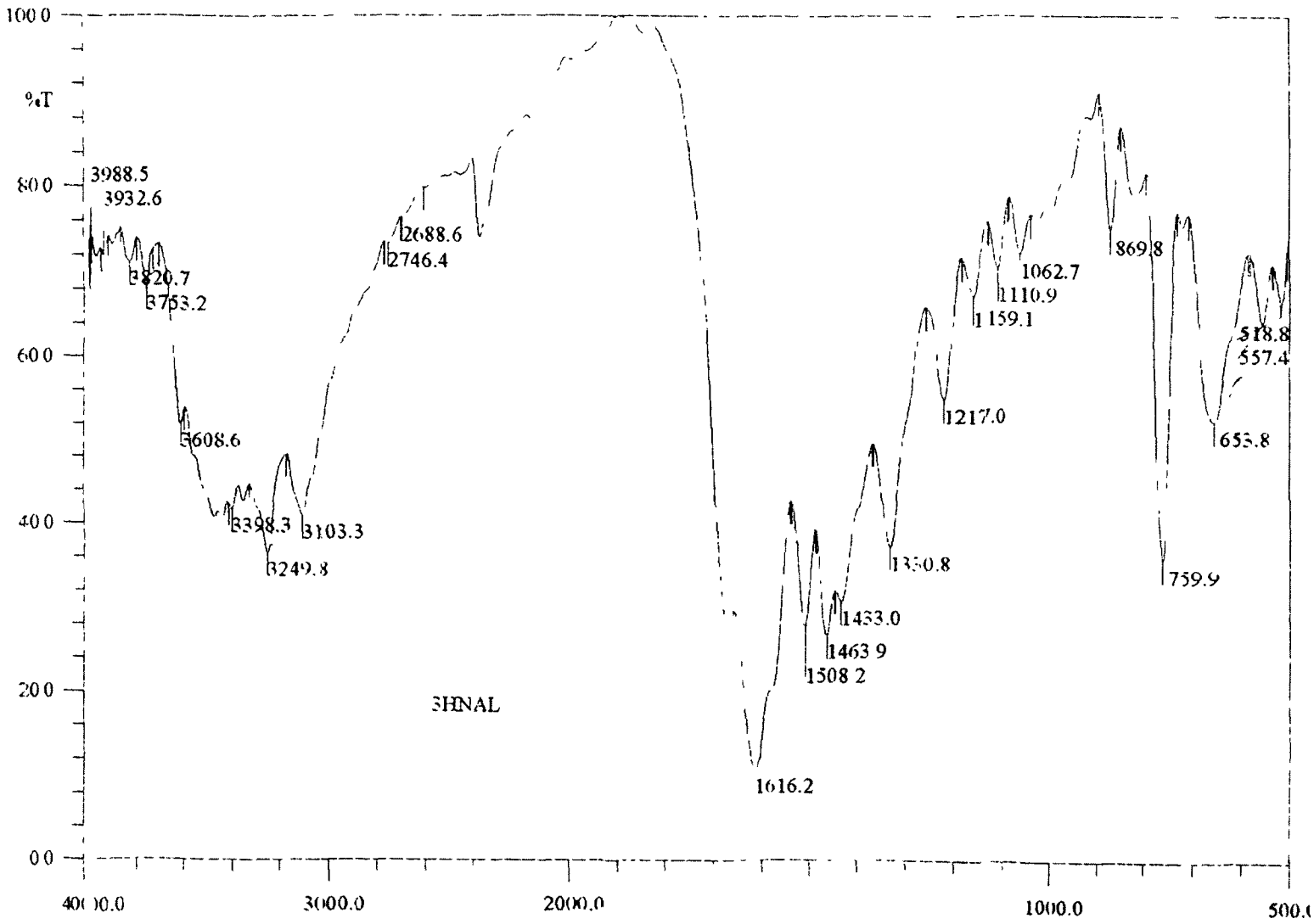
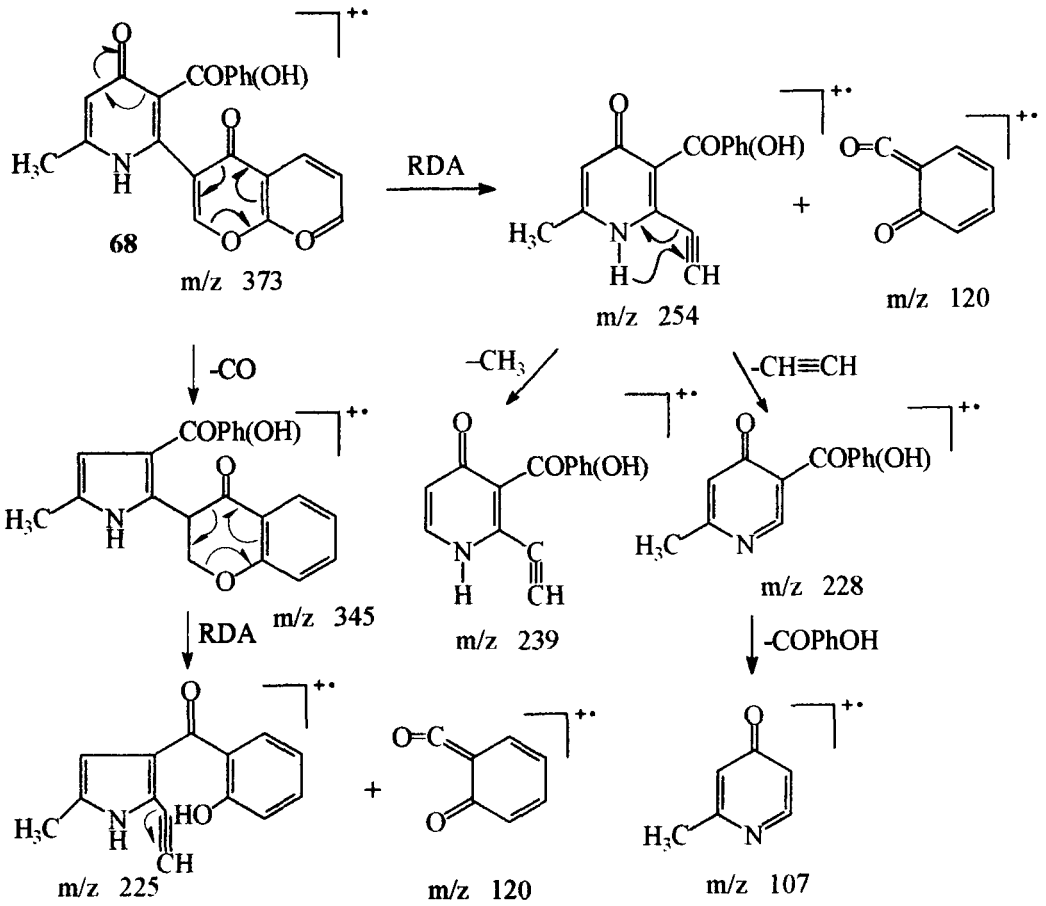
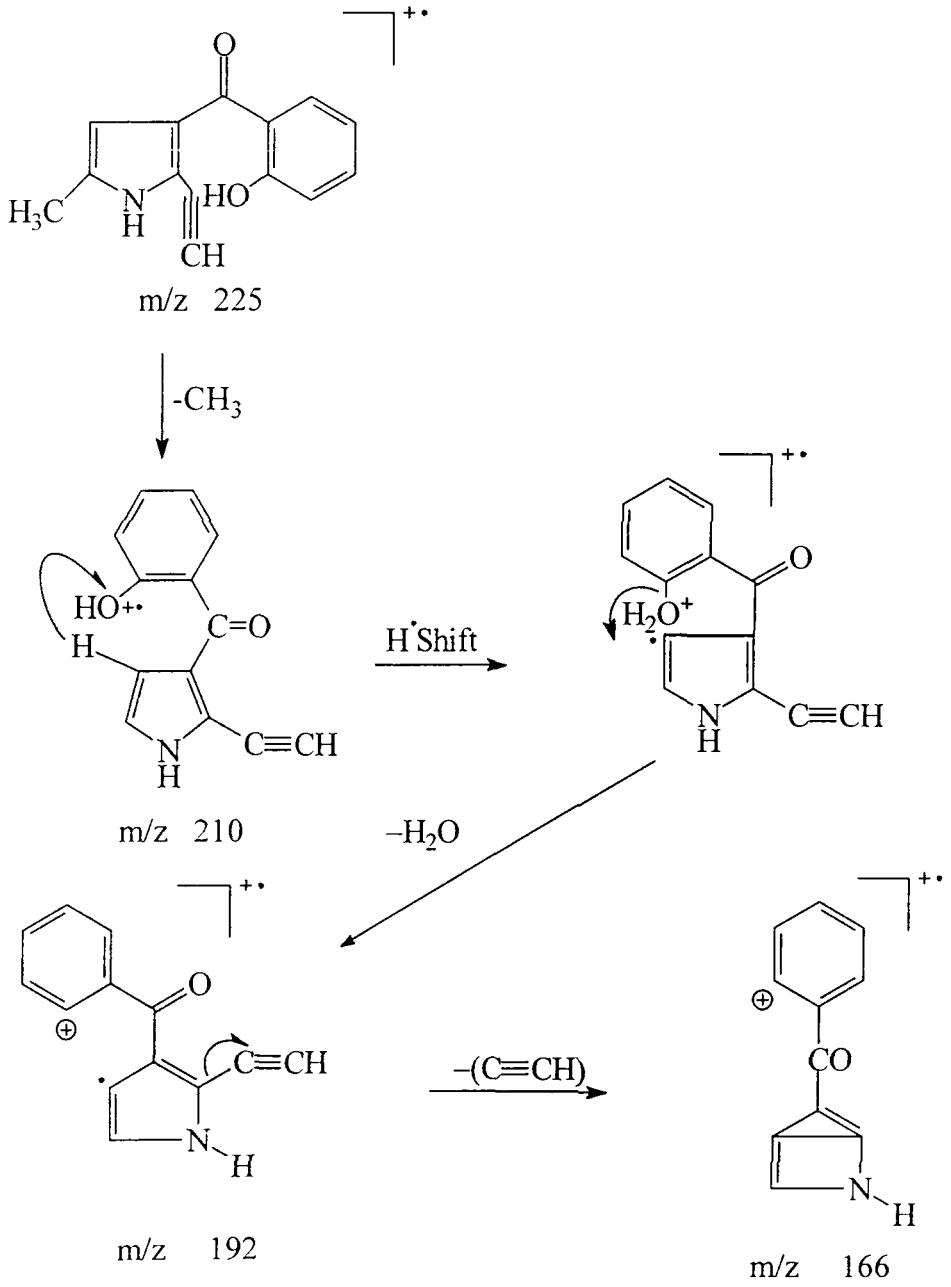

100 0
%T
80 0
60 0
40 0
20 0
00
3988.5
3932.6
3820.7
3753.2
3608.6
3398.3
3249.8
3103.3
2688.6
2746.4
1616.2
1508 2
1463 9
1433.0
1350.8
1217.0
1159.1
1110.9
1062.7
869.8
759.9
655.8
557.4
518.8
3HNAL
400 0.0
3000.0
2000.0
1000.0
500.0

Fig. 27

δ 7.07 thus has been assigned to C-5 proton. The presence of chromone moiety is established by C'-5 proton in the forms of ortho coupled doublet at δ 8.02 (J=9 Hz). The C'-2 proton of chromone unit is clearly shown as a sharp singlet at δ 8.61. A slightly broad singlet at δ 9.58 and a broad singlet at δ 16.68 (both D_2O exchangeable) have been assigned to NH and OH protons respectively. Mass spectrum (Fig. 25) further supports structure **68** for the compound showing M^+ at m/z 373. The loss of carbonyl group from M^+ gives peak at m/z 345. The peak at 345 undergoes RDA cleavage followed by loss of methyl group and a water to give base peak at m/z 192. The other peaks are obtained as outlined in Scheme 32.



Scheme 32



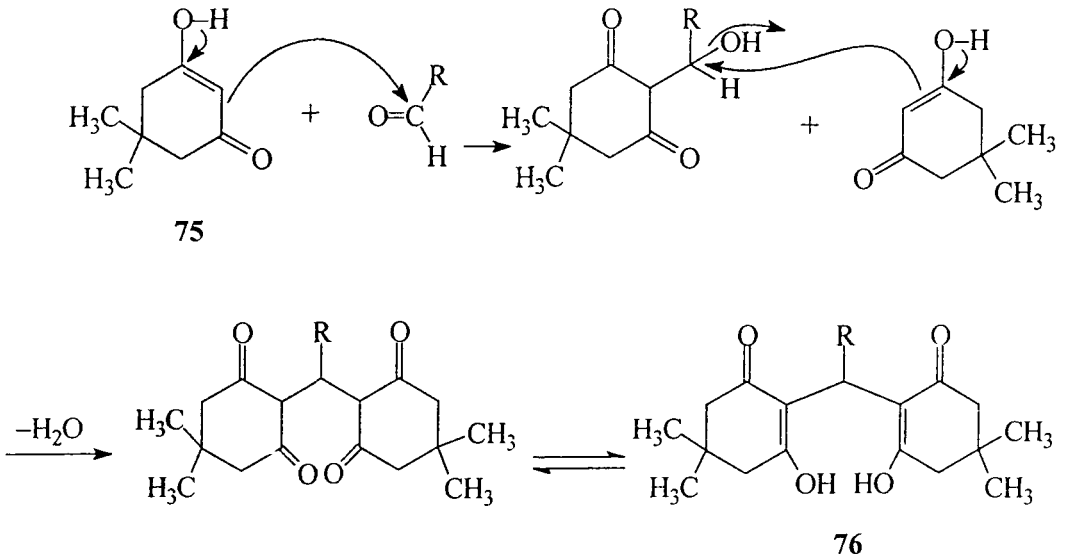
Scheme 32

3.5 The reaction of 2-amino-3-formylchromone 50 with active methylene Compounds

3.5.1 The reaction of 2-amino-3-formylchromone 50 with 5,5-dimethyl cyclohexene-1,3-dione

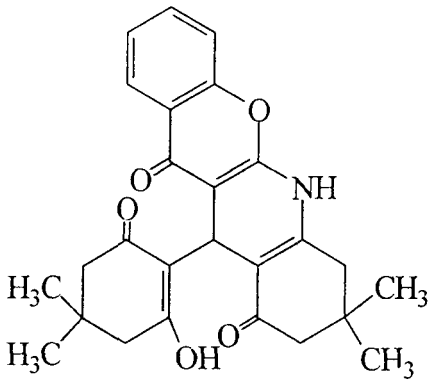
Azaxanthones are heterocycles of pharmacological interest. They have been used as bronchodialators[63,64] and antiallerygic[65] agents. It appeared, therefore, interesting to synthesize azaxanthones from the reaction of 2-amino-3-formylchromone **50** with 5,5-dimethyl cyclohexanes 1,3-dione **75** and 3-methyl-1-phenyl-5- pyrazolone **79** under mild conditions.

Dimedone, **75** is known to form crystalline compound **76** with aldehydes[66] and triethyl orthoformat[67] (Scheme 33).

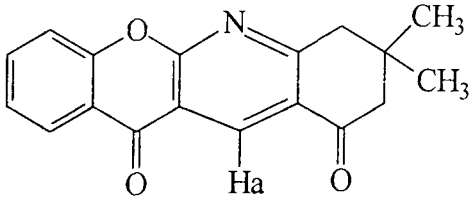


Scheme 33

Since 2-amino-3-formylchromone **50** has CHO group it should give **77** upon treatment with **75**. The compound, however, did not give any colour with ferric chloride. Further mass spectrum (Fig. 28) was also not in agreement with expected structure **77**. It showed M^+ at rather low value i.e. at m/z 294 (M+1 peak) which again ruled out the possibility of dimeric structure **77**.



77



78

The compound shows strong IR bands (Fig. 29) at 1685 and 1651 cm^{-1} for chromone and α, β-unsaturated ketone carbonyl groups. A strong band which appears at 1595 cm^{-1} is due to C=N bond. The ^{1}HNMR spectrum (Fig. 30) shows a sharp singlet integrating for 6 protons at δ 1.17 and has been assigned to two methyl groups. To more singlets at δ 2.64 and 3.14, each integrating for 2 protons are given to the methylene groups of dimedone unit. These features indicates participation of only one dimedone moiety in the formation of the product. The presence of intact

[Mass Spectrum]
Data : 5ESEP23608 Date : 23-Sep-2005 11:09
Sample: 3HNAD DR ZEBA N SIDDIQUI,ALIGARH #8935
Note : -
Inlet : Direct Ion Mode : FAB+
Spectrum Type : Normal Ion [MF-Linear]
RT : 0.73 min Scan# : (6,8)
B.P : m/z 294.0000 Int. : 58.03
Output m/z range : 104.5252 to 652.1289 Cut Level : 0.00 %

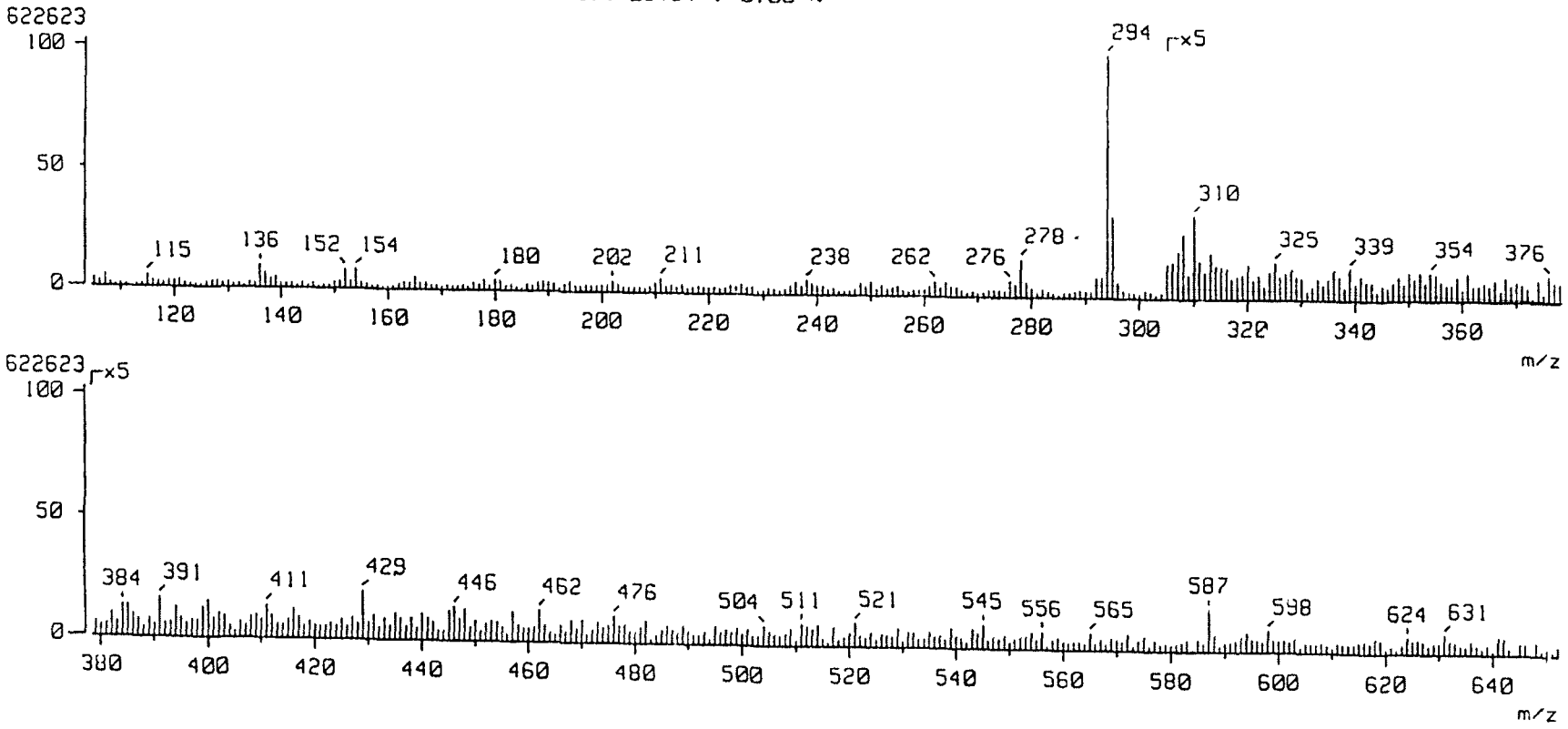
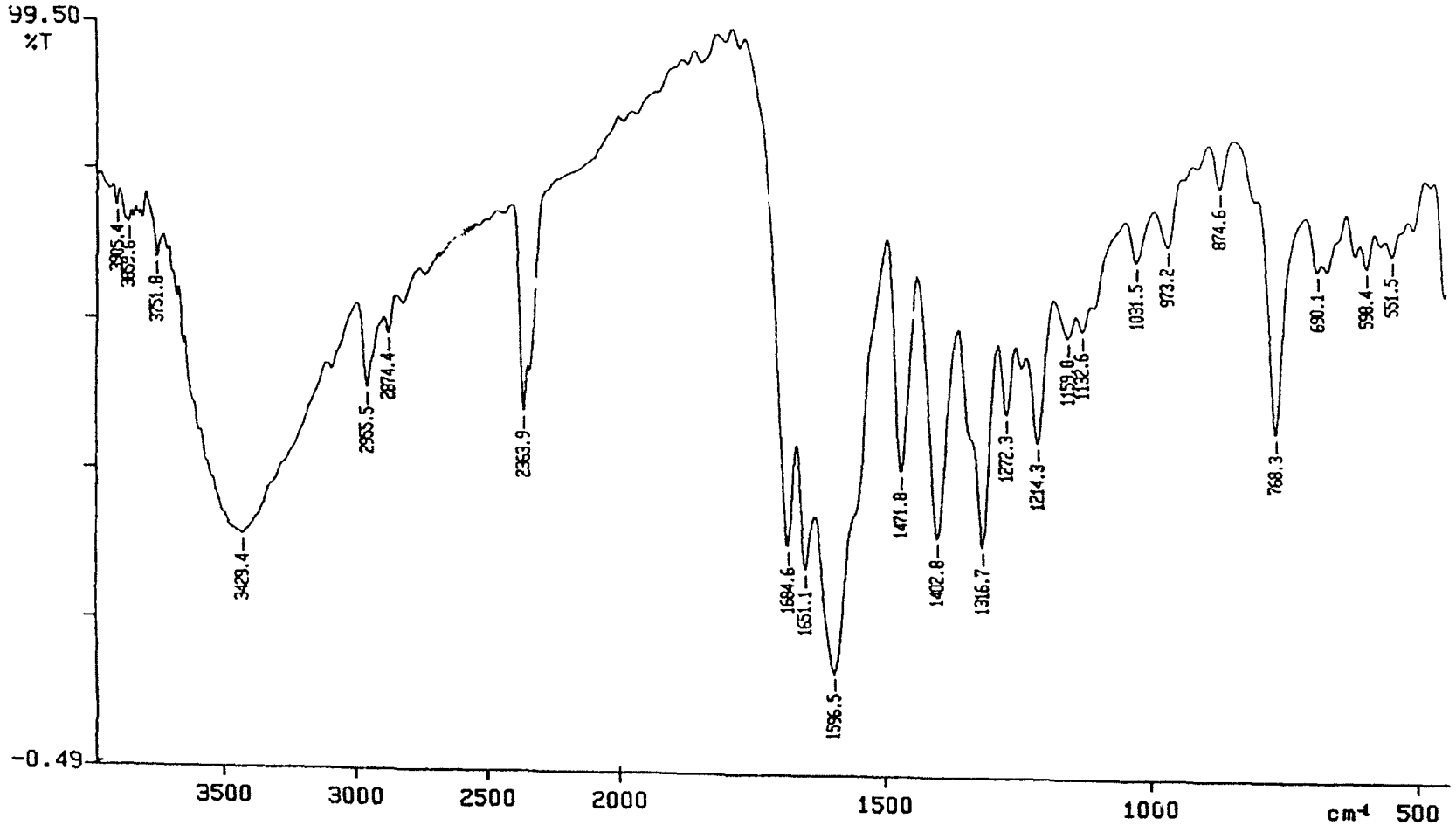


Fig. 28



05/09/21 10:57 AH. CODE:
X: 4 scans, 4.0cm-1, flat, smooth, abex

Fig. 29

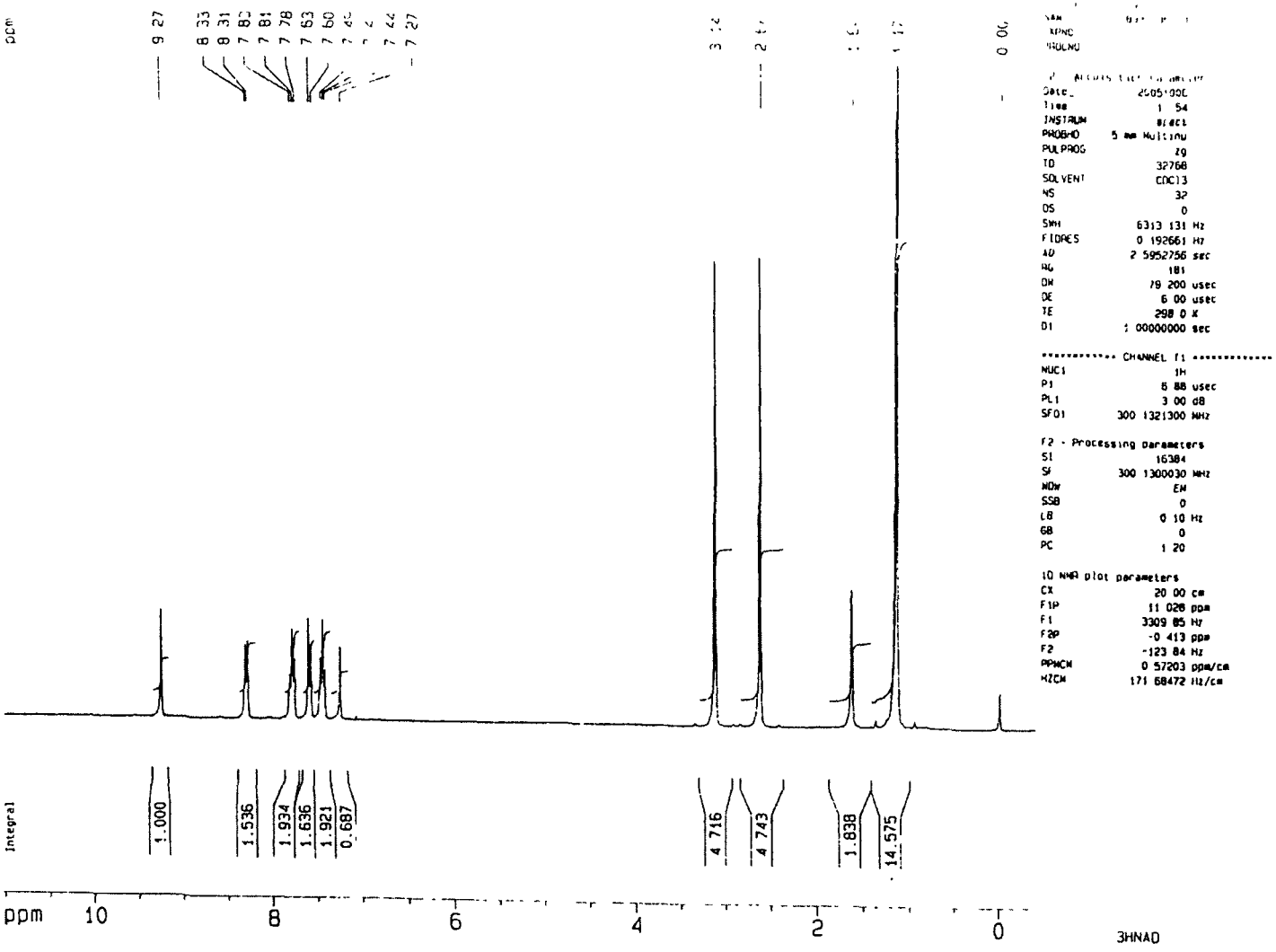
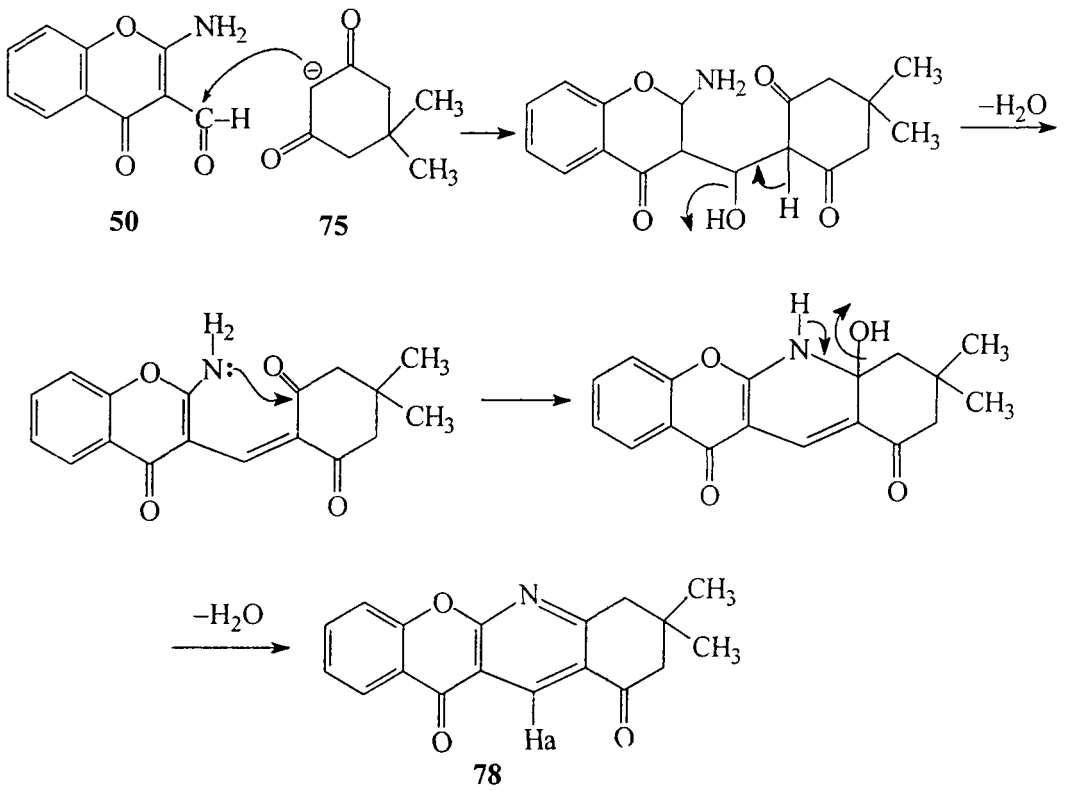

ppm
9.27
8.33
8.31
7.83
7.81
7.78
7.63
7.60
7.44
7.27
3.74
2.67
1.57
1.17
0.00
Integral
1.000
1.536
1.934
1.636
1.921
0.687
4.716
4.743
1.838
14.575
ppm
10
8
6
4
2
0
3HNAD
Date_ 25051998
Time 1 54
INSTRUM
PROBHD 5 mm Multinu
PULPROG zg
TD 32768
SOLVENT CDCl3
NS 32
DS 0
SWH 6313 131 Hz
FIDRES 0 192661 Hz
AQ 2 5952756 sec
RG 181
DW 79 200 usec
DE 6 00 usec
TE 298 0 K
D1 1 00000000 sec
CHANNEL f1
NUC1 1H
P1 8 88 usec
PL1 3 00 dB
SFO1 300 1321300 MHz
F2 - Processing parameters
SI 16384
SF 300 1300030 MHz
WDW EM
SSB 0
LB 0 10 Hz
GB 0
PC 1 20
1D NMR plot parameters
CX 20 00 cm
F1P 11 028 ppm
F1 3309 85 Hz
F2P -0 413 ppm
F2 -123 84 Hz
PPMCM 0 57203 ppm/cm
HZCM 171 68472 Hz/cm

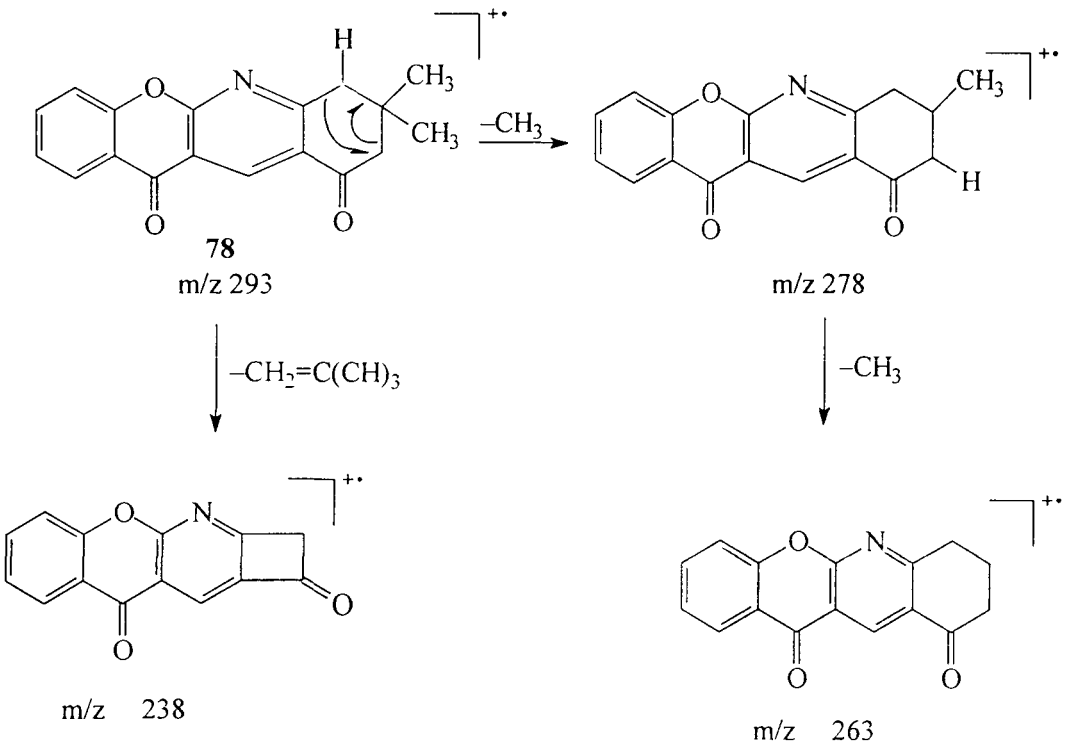
Fig. 30

chromone moiety is evident from the doublet of C-5 proton of chromone moiety at δ 8.33. The remaining three hydrogens of chromone nucleus give multiplets situated between δ 7.44-7.83. The most down field singlet at δ 9.27 is assigned to H_a proton which is peri to two carbonyl groups. Combining these spectral data one arrives at structure **78** for the compound and its formation can be rationalized as shown in scheme 34.



Scheme 34

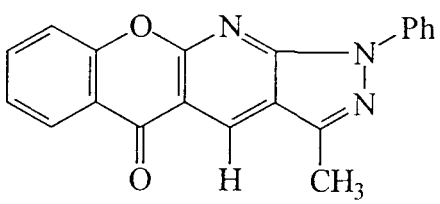
Further confirmation for the compound is provided by its mass spectrum (Fig. 28) showing M^+ at 294 (M+1) peak) which is also the base peak. Other peaks are obtained as shown in Scheme 35.



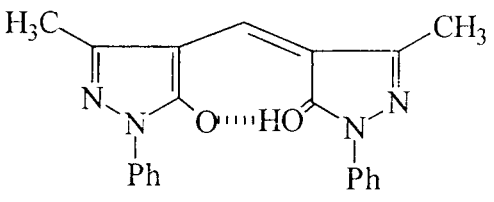
Scheme 35

The same compound **78** was synthesized by Helmut et al[68]. by refluxing of the starting materials **50** and **75** under strong basic conditions whereas we have synthesized **78** at room temperature with improved yield (90%). Further we have explored different types of biological activities which are discussed in the last chapter.

3.5.2 The reaction of 2-amino-3-formylchromone with pyrazolone



80



81

Attempts were directed towards the synthesis of another azaxanthone, viz. pyrrazoloazaxanthone **80** by conducting the reaction of 2-amino-3-formylchromone with 3-methyl-1-phenyl-5-pyrazolone **79** under different reactions conditions. Under mild acidic conditions employing catalytic amount of p-toluene sulphonic acid or glacial acetic acid the reaction mixture, however, afforded unexpected and dimeric product **81.** Under mild basic conditions using minute quantity of pyridine or sodium/potassium acetate again the dimer **81** was obtained. The ease with which the compound **81** is obtained in all acidic and basic conditions seems due to the formation of intermediate, formyl derivative of pyrazolone **82** which undergoes dimerization by attack of another molecule of pyrazolone **79** (Scheme 36).

The compound gave light blue-black colour with ferric colour.

The IR spectrum (Fig. 31) of **81** is devoid of any chromone carbonyl group possibility of chromone nucleus in the compound is, thus, ruled out. It displays a broad band for OH group at 3414 cm^{-1} and a strong band at 1628 cm^{-1} for α,β-unsaturated carbonyl group. The value of carbonyl group is somewhat lower than that mentioned in literature[69]. This discrepancy in the frequency may be due to intramolecular H-bonding in the molecule which shifts the IR frequency for carbonyl group towards longer wavelength. The ^1H NMR (Fig. 32) shows a sharp singlet integrating for six proton at δ 2.32 due to the presence of two

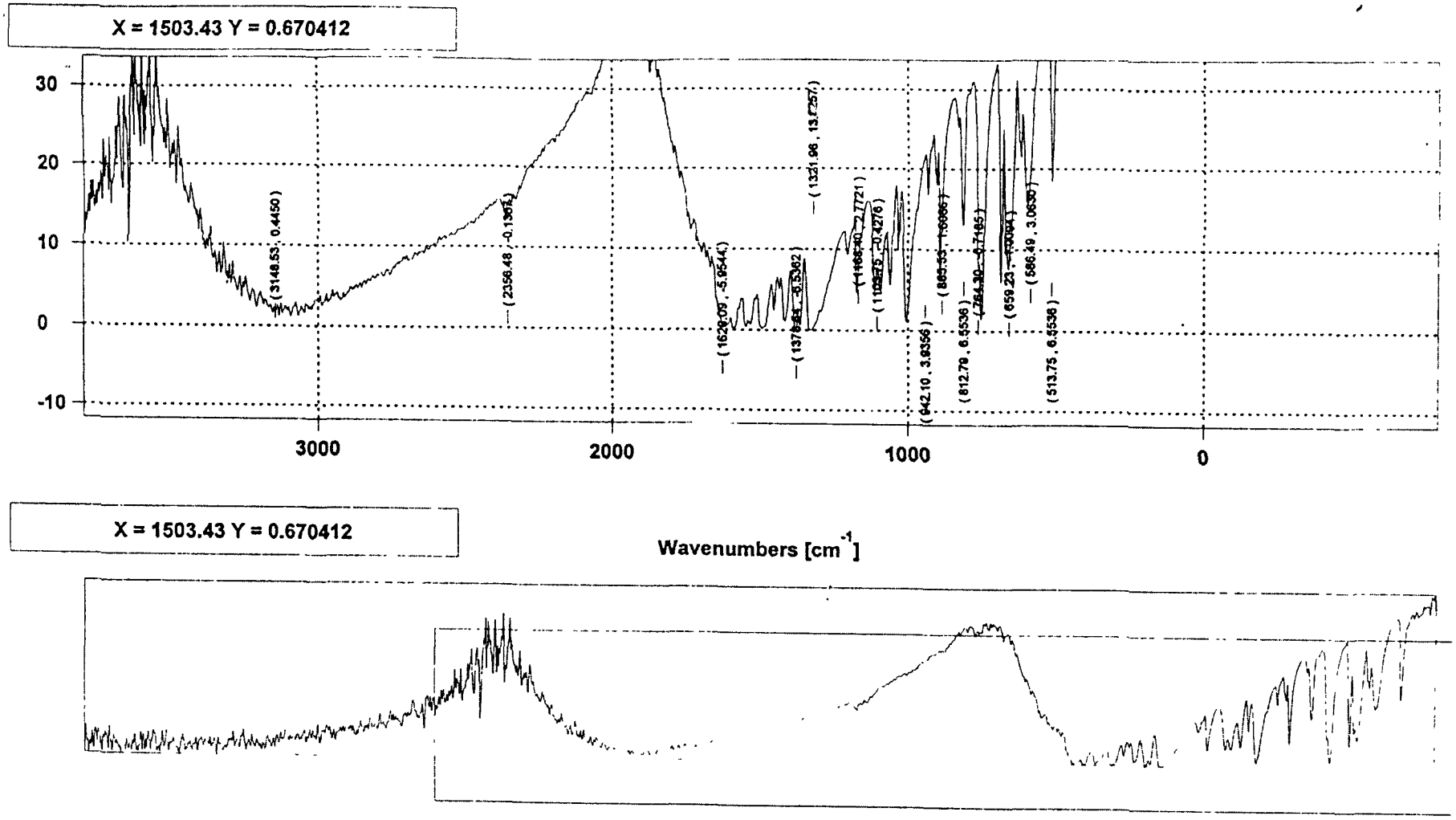

X = 1503.43 Y = 0.670412
30
20
10
0
-10
3000
2000
1000
0
X = 1503.43 Y = 0.670412
Wavenumbers [cm-1]

Fig. 31

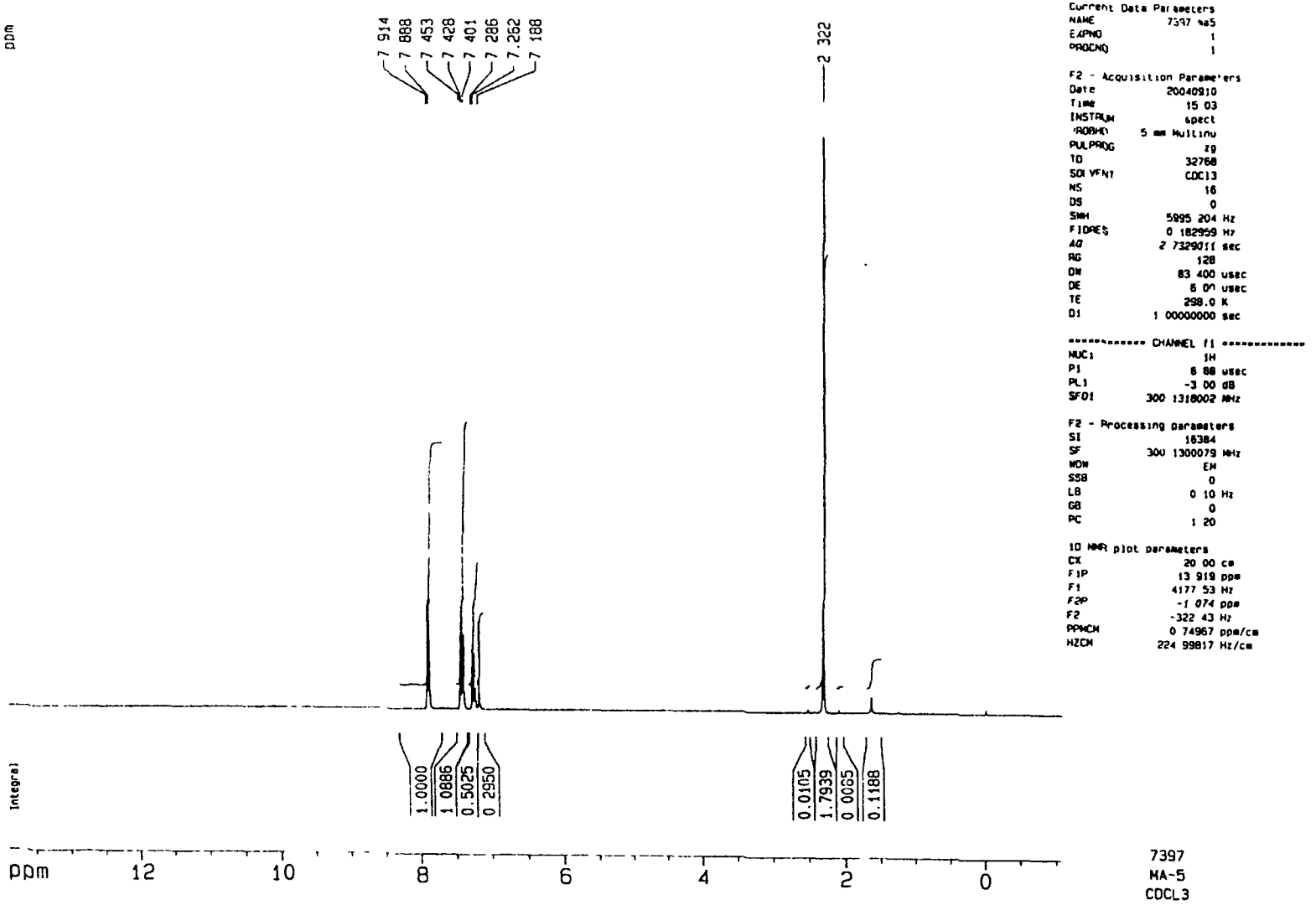

ppm
7.914
7.888
7.453
7.428
7.401
7.286
7.262
7.188
2.322
Integral
1.0000
1.0886
0.5025
0.2950
0.0105
1.7939
0.0065
0.1188
ppm
12
10
8
6
4
2
0
Current Data Parameters
NAME 7397 ma5
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date 20040910
Time 15 03
INSTRUM spect
PROBHD 5 mm Multinu
PULPROG zg
TD 32768
SOLVENT CDCl3
NS 16
DS 0
SWH 5995.204 Hz
FIDRES 0.182959 Hz
AQ 2.7329011 sec
RG 128
DW 83.400 usec
DE 6.00 usec
TE 298.0 K
D1 1.00000000 sec
CHANNEL f1
NUC1 1H
P1 8.88 usec
PL1 -3.00 dB
SFO1 300.1318002 MHz
F2 - Processing parameters
SI 16384
SF 300.1300079 MHz
WDW EM
SSB 0
LB 0.10 Hz
GB 0
PC 1.20
1D NMR plot parameters
CX 20.00 cm
F1P 13.919 ppm
F1 4177.53 Hz
F2P -1.074 ppm
F2 -322.43 Hz
PPMCM 0.74967 ppm/cm
HZCM 224.99817 Hz/cm
7397
MA-5
CDCL3

Fig. 32

[Mass Spectrum]
Data : 4EAGU574 Date : 30-Aug-2004 16:00
Sample: MA-5 DR[MRS] ZN SIDDIQUI,ALIGARH #7397
Note :
Inlet : Direct Ion Mode : FAB+
Spectrum Type : Normal Ion [MF-Linear]
RT : 0.12 min Scan# : (1,3)
BP : m/z 359.0000 Int. : 28.14
Output m/z range : 71.8101 to 504.3769 Cut Level : 0.00 %

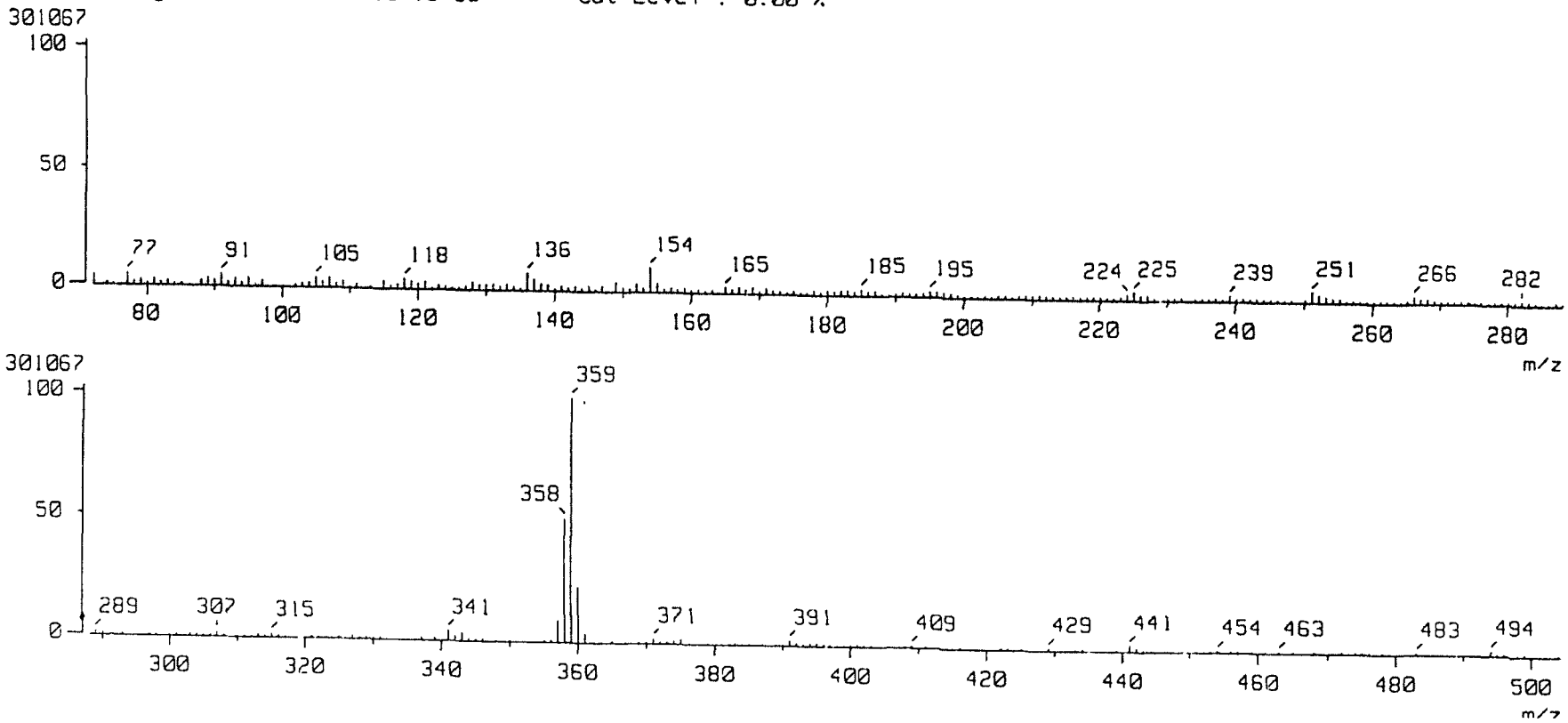
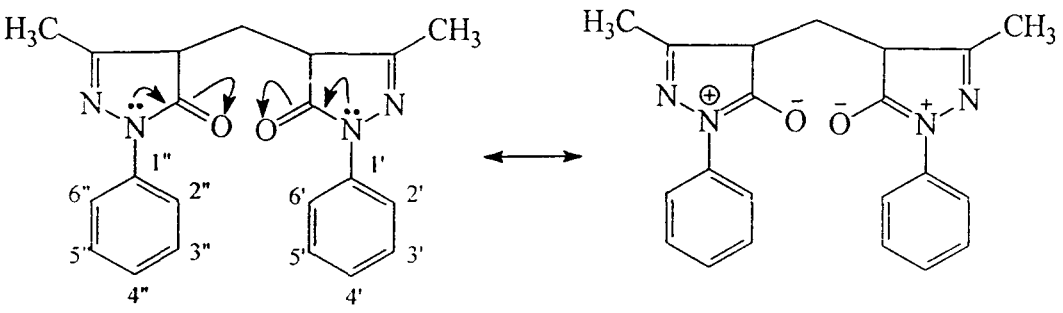
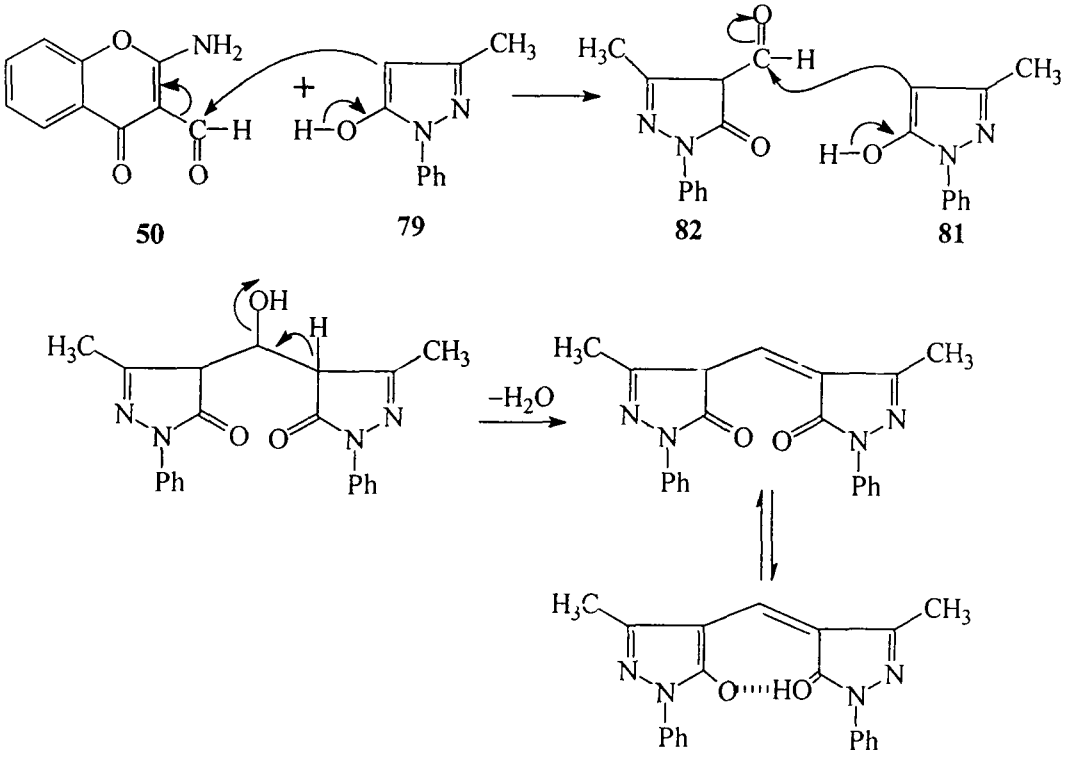


Fig. 33

methyl group in the compound whereas **80** if assigned to the compound, has only one methyl group. Further mass spectrum (Fig. 33) which shows M^+ at 359 (M^++1) (peak), is also not compatible with structure **80** and requires odd molecular ion peak instead of even here. The NMR spectrum completely eliminates structure **80** as it does not show any doublet for C-5 proton of chromone moiety and shows a sharp singlet of olefinic proton at δ 7.18. The aromatic region of the spectrum shows ten aromatic protons of two benzen rings in the form of two mutliplets, situated at δ 7.23-7.28 integrating for two protons, δ 7.40-7.45, integrating for four protons and in the form of two singlets at δ 7.88 and 7.91 respectively each integrating for two protons. The down field singlets of four protons of two benzen rings may be due to presence of carbonyl group adjacent to N so that withdrawal by carbonyl group makes nitrogen positively charged which shifts adjacents C-2', C-6' and C-2", C-6" protons, in much down field region





Scheme 36

The compound **81** was obtained by Wallace et al. while synthesizing 4-formyl-3-methyl-1-phenyl 5-pyrazole, **82**[70].

CHAPTER 2

Isolation and Structure Determination of Natural Products From *Piper cubeba* and *Zanthoxylum simularis*

2.1 Introduction

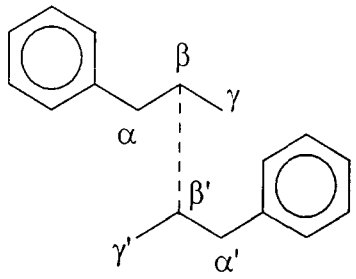
The work discussed in this chapter is based on the investigation of medicinally important plants *Piper cubeba, Piper chaba* and *Zanthoxylum simularis* of families *Piperaceae* and Rutaceae respectively. The investigation of these plants was taken up because of the medicinal properties attributed to it and related species. In the present investigation efforts were directed towards isolation of compounds from these plants. Attempts to separate the components through preparative TLC did not succeed because R_f values of the components were very close. Alternatively, column chromatography was then employed for resolution of the mixture of crude extracts. The compounds resolved were subjected to detailed spectroscopic analysis. This resulted in their identification as cubebin from *Piper cubeba*, limonin and 4'5-dihydroxy-6", 6"-dimethylpyrano (2",3",7,6) flavone from *Zanthoxylum simularis*.

The investigation of *Piper chaba* commonly known as Kankal was taken up because lignans[71], amides[72] and novel alkaloids[73] have been reported from this and other species of genus Piper. Acetone extract of *Piper chaba* yielded a compound which was labelled as PchAc3. The spectral characteristic of PchAc3, melting point 240° and positive Shinoda's test (Mg+HCl) indicated it to be a hydroxy flavone (presence of OH group in poorly resolved ^{1}H NMR). Owing to paucity of the

compound in hand and the difficulty in getting exhaustive spectral data, its structure could not be established so for.

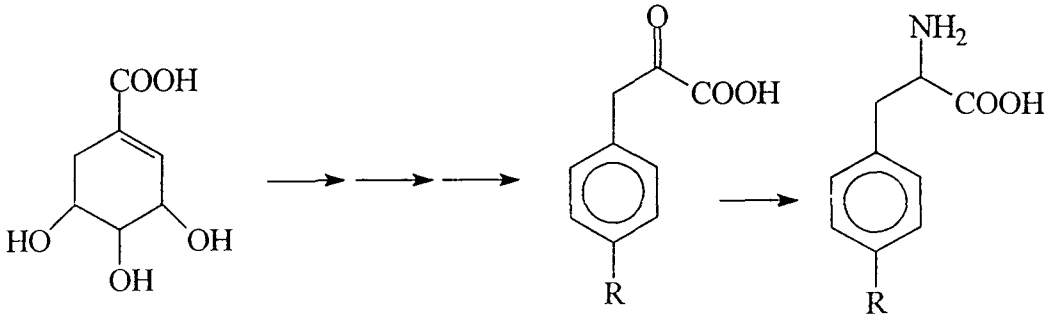
2.2 Theoretical:

Lignans are naturally occurring compounds in which two phenyl propane units are joined together from 2 and 2' positions by atleast one carbon-carbon bond **83**.

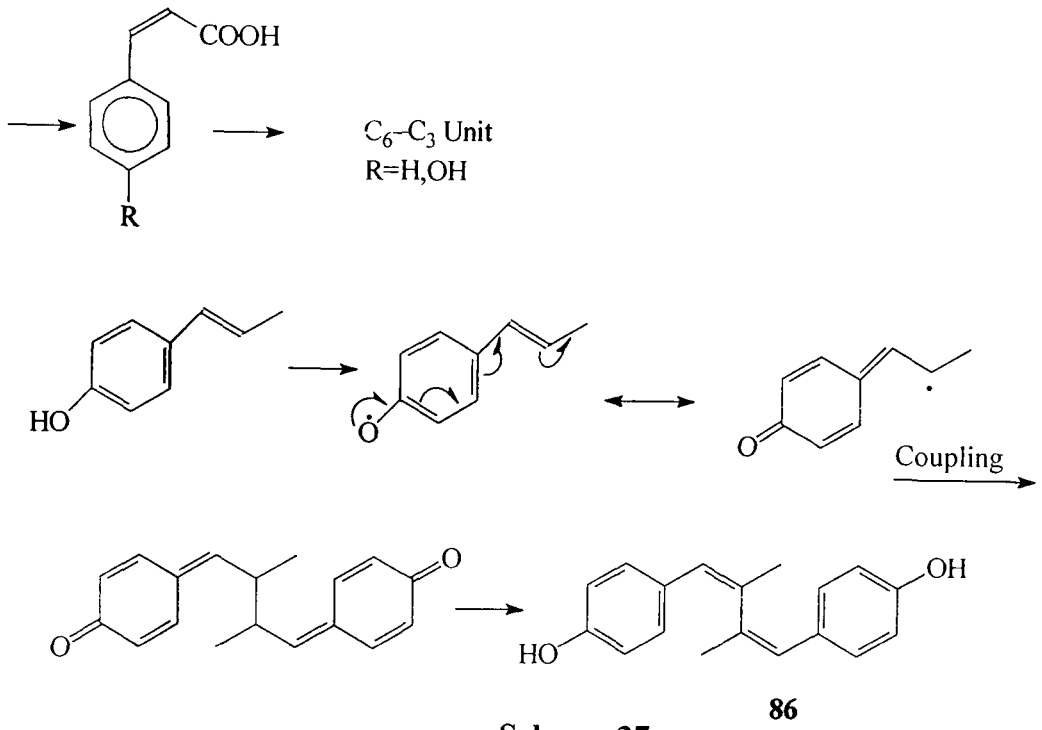


83

Two benzene rings are usually identically substituted. The type of substitution and nature of substituants is similar to that in flavonoids. Both phenyl propane units are generally derived from shikimic acid as framed by Robinson[74] and C-C bond is created as a result of oxidative coupling of two trans propenyl benzene units (Scheme-37).



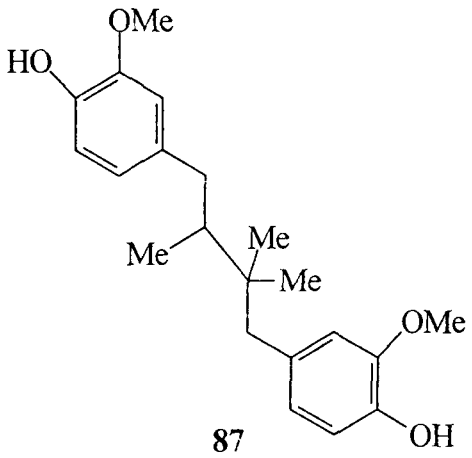
84 **85**



86

Scheme 37

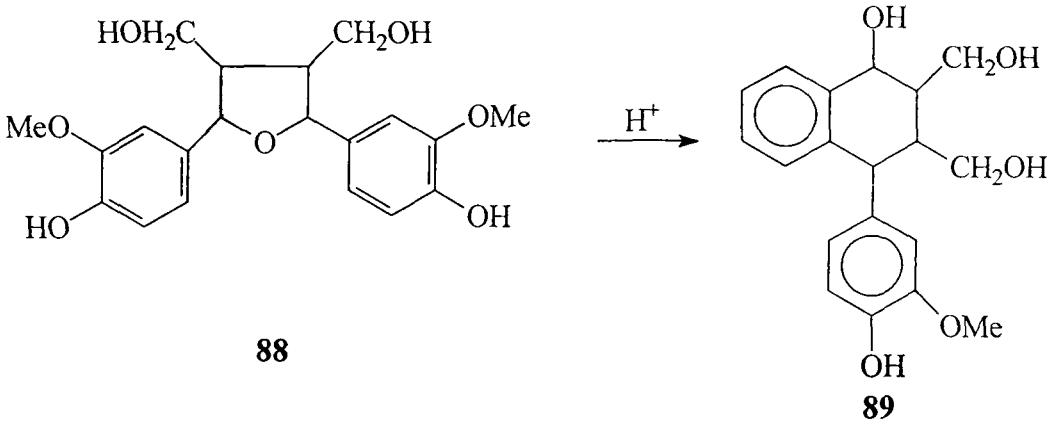
First shikimic acid **84**. is transformed to amino acid **85** in plants which, forms C_6-C_3 fragment by removal of ammonia. Two such C_6-C_3 units, then undergo oxidative coupling when new β-β' bond in **86** is created.



87

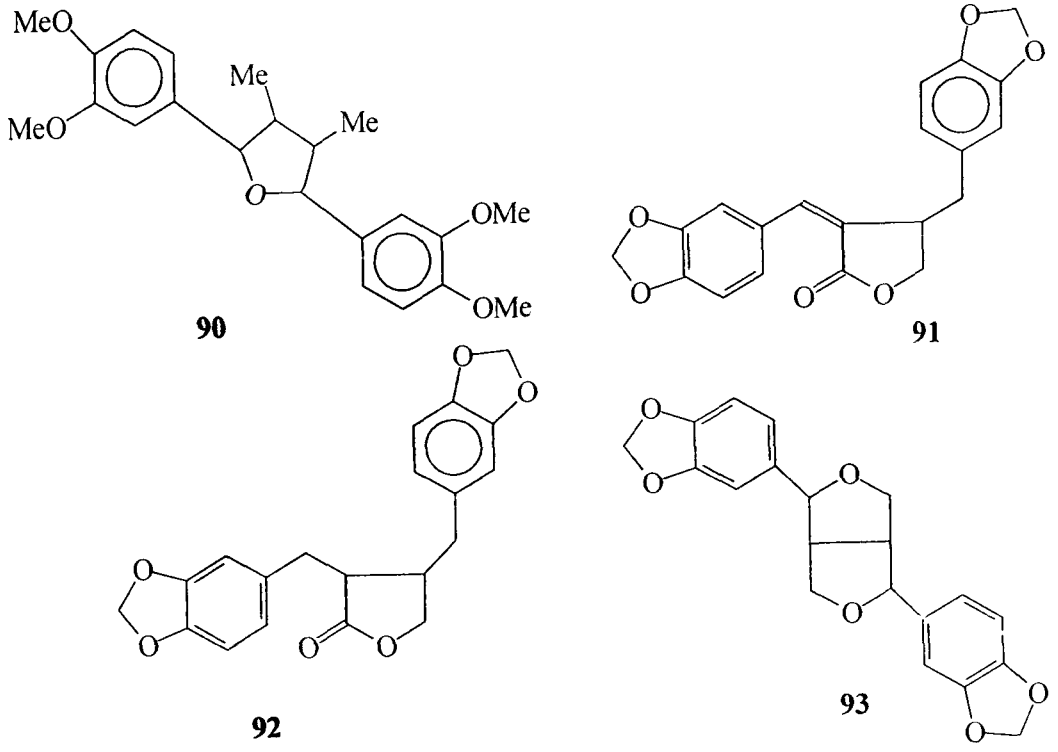
Various types of lignans are known, the simplest one being guaiaretic acid **87**. Important ones are those which have a tetrahydrofuran ring in their structures. In this class of naturally occurring compound, olivil **88** is the first lignan, characterized by Vanzett[75]. The compound **88**,

isolated from *Olea europia*, is converted to its isomer **89** through ring opening of furan ring under acidic conditions.



88 **89**

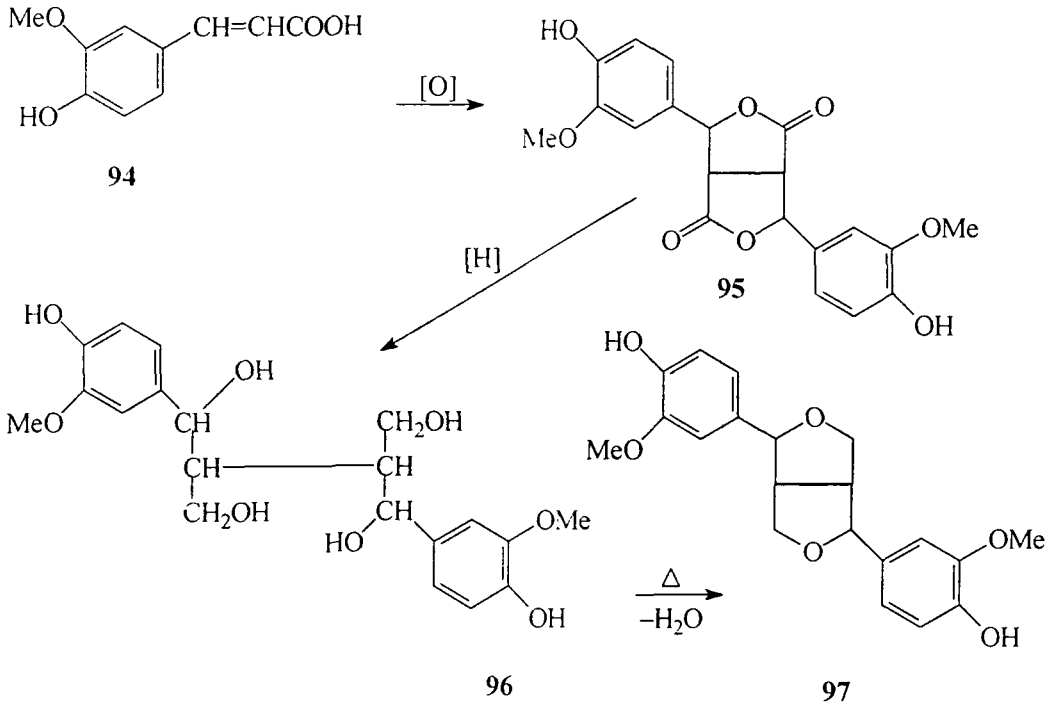
Other tetrahydrafuran lignans are galgravin[76] **90** isolated from *Himantandra belgraveana*, savinin[77] **91,** which also contains methylene dioxy substituted benzene ring has been isolated from *Juniperus sabina* hydrogenation of which gives hinokinin **92** and sesamin[78,79] **93** from *Asarum sieboldii.*



90 **91**

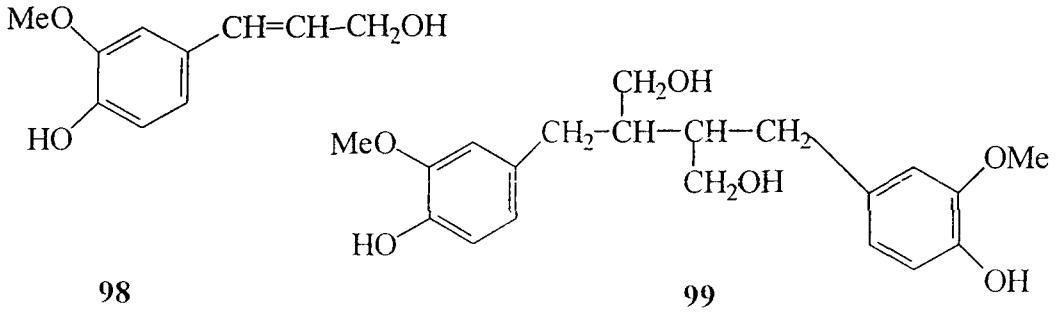
92 **93**

Other type of lignans in which two furan rings are joined together from opposite sides include dilactone **95** which is formed by oxidation of ferulic acid **94** in the presence of ferric ions through electron transfers[80]. Upon reduction and subsequent ring opening, **95** is transformed to polyhydric alcohol **96** which is recyclized, on distillation, to pinoresinol **97**, which is a major constituent of *Pinus lavicio*[81] (Scheme 38).



Scheme 38

Similarly corniferyl alcohol **98** which also occurs in the cambial sap of plants undergoes oxidation in the presence of enzymes and forms dehydrodiconiferyl alcohol **99** and pinoresinol **97**[81].



Enzymatic oxidation also forms lignin like material and this gives an idea that lignans are building units in the formation of lignin which provides toughness to the plant tissues[82].

2.3 Discussion

2.3.1 Chemical constituents of Piper cubeba

Piper cubeba (Piperaceae), a medicinally important plant, is commonly known as Kabab chini and cubebs. It was collected from Mysore, India. A survey of literature of ~30 years has shown that most of the compounds isolated from *Piper cubeba* are lignans[83-85]. Besides these, oxygenated cyclohexanes[86] and volatile oils[87] have also been isolated. Other species of genus *Piper*, however, have produced novel alkaloids[88,89], neolignans[90], terpenes[91] and flavonoids[92].

In the context of present work an attempt was made to isolate and identify active principles from this plant. Crude extract of the plant showed a number of compounds on TLC plates. Efforts were made to separate these compounds through column chromatography. This led to isolation of a single compound in major quantity. Structure of the compound, labelled as KCB, was inferred through spectroscopic data, evaluated for its possible biological properties and is discussed in this chapter.

The plant material was successively extracted with solvents of increasing polarity. Purification was effected by column

chromatography over silica gel. Elution of the column with benzene ethyl acetate yielded a white crystalline solid which was labeled as KCB.

The IR spectrum (Fig. 34) of the compound, melting point 120-22°C is devoid of any carbonyl group, in the region 1630-1750 cm^{-1}, possibility of coumarin or flavonoid derivative is, thus, ruled out. It also does not show any upfield methyl singlets in the nmr spectrum (Fig. 35) which suggests that the compound is also not a terpene. The spectral characteristic, however, indicates it as lignan. In the IR spectrum a broad band at 3418 cm^{-1} indicates presence of a hydroxyl group. Other IR bands at 1629 and 926 cm^{-1} are due to carbon-carbon double bonds and methylene dioxy functional groups. In ^{1}H NMR spectrum the aromatic region shows two multiplets integrating for six protons at δ 6.61-6.81. This shows the presence of two benzene rings in the compound. A sharp singlet at δ 5.95 integrating for four protons has been assigned to two methylenedioxy groups attached to different benzene rings. A fine triplet for one proton which appears at δ 5.03 may be assign to a proton which is under the influence of two oxygen atoms. A multiplet in the region δ 3.81-3.86 may be assigned to methylene protons under oxygen. In addition to these features, the spectrum also shows a broad singlet (D_2O exchangeable) at δ 3.50 and can be assigned to a hydroxylic proton. The multiplet at δ

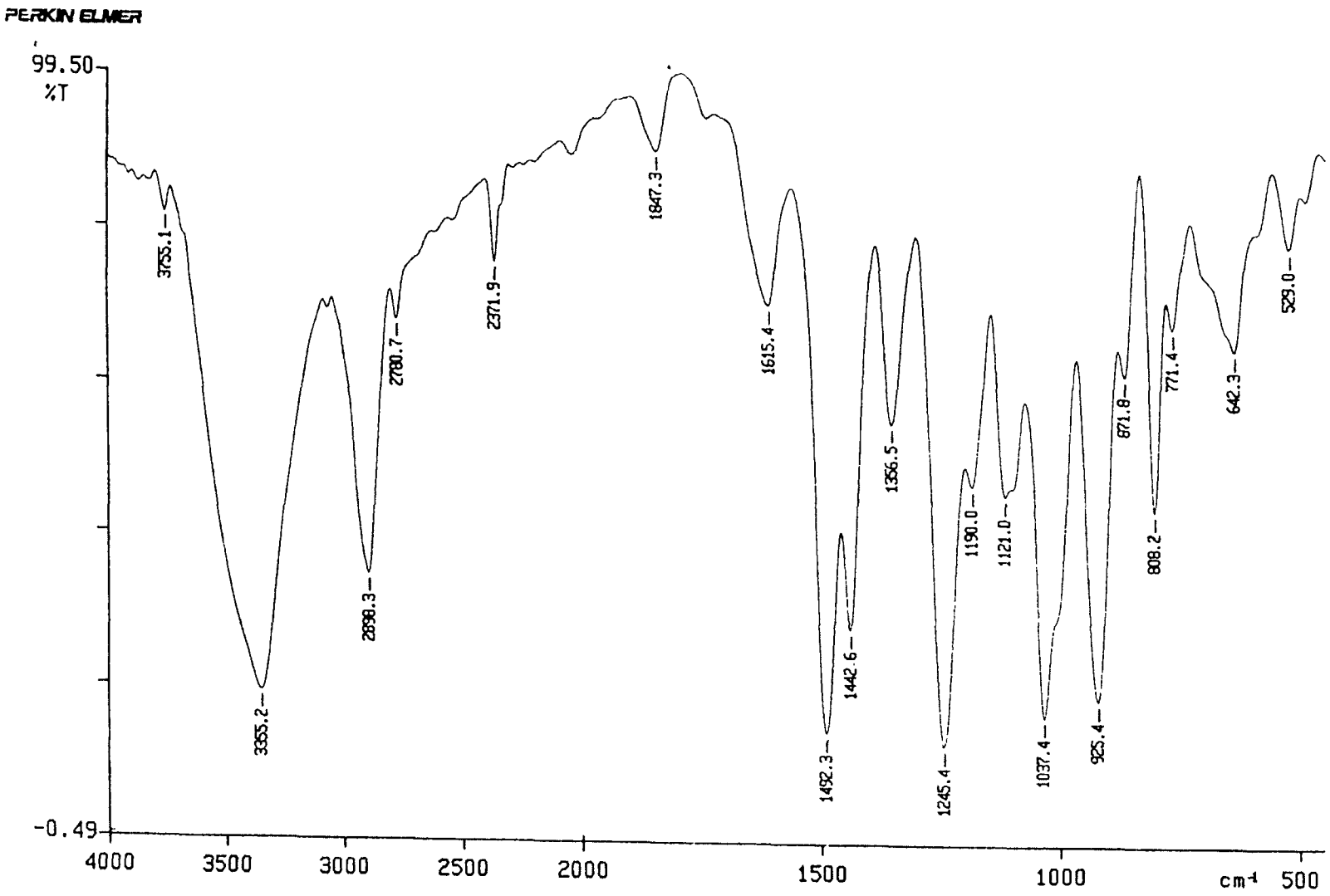

PERKIN ELMER
99.50
%T
-0.49
3755.1
3355.2
2890.3
2780.7
2371.9
1847.3
1615.4
1492.3
1442.6
1356.5
1245.4
1190.0
1121.0
1037.4
925.4
871.8
808.2
771.4
642.3
529.0
4000
3500
3000
2500
2000
1500
1000
cm-1
500

Fig. 34

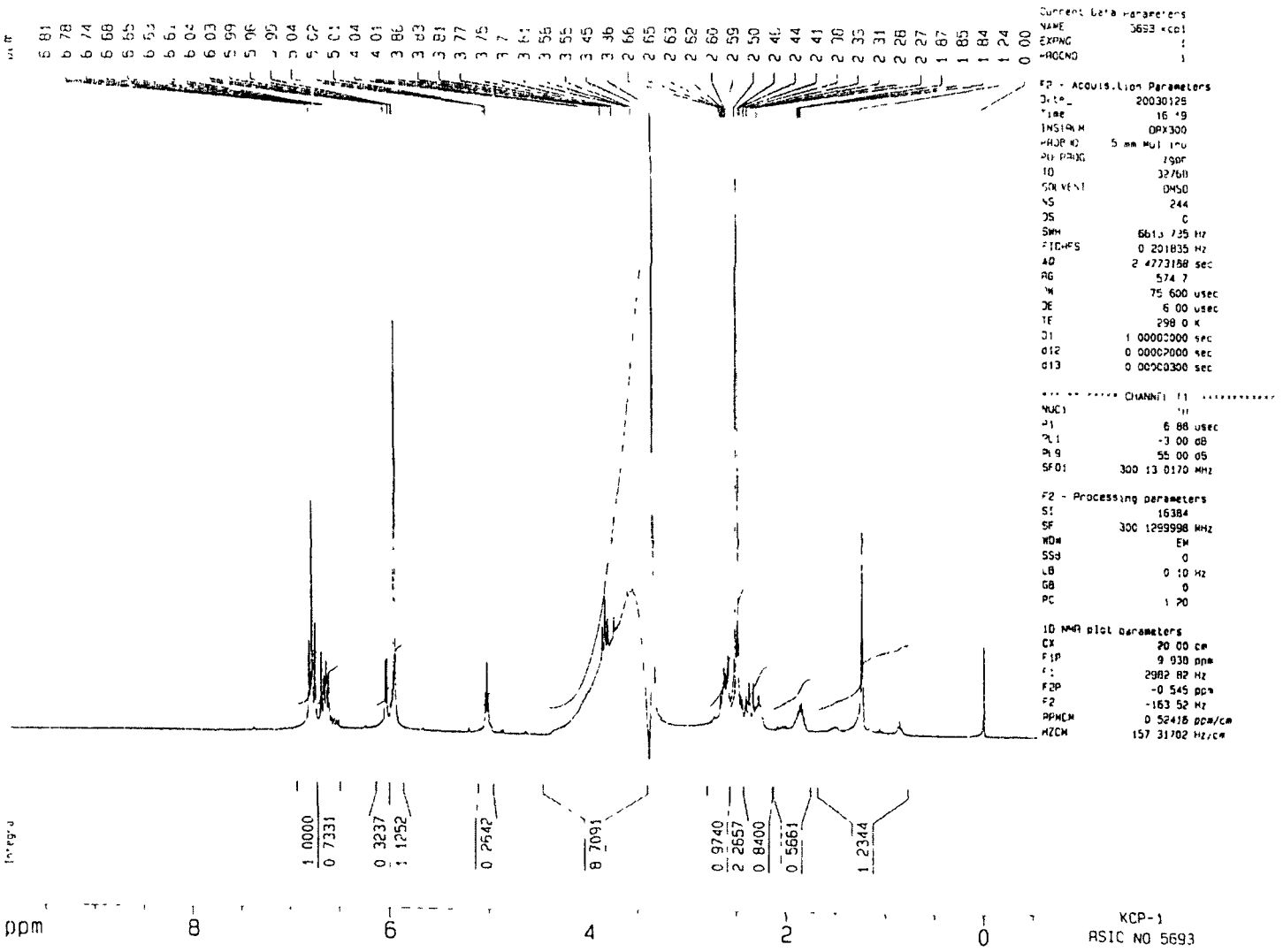
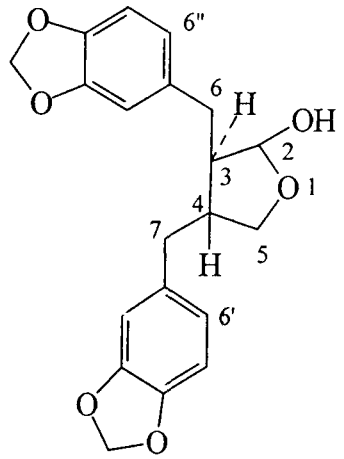


Fig. 35

1.80-2.66 integrating for six protons has been assigned to benzylic and methine protons.

Combining these spectral feature one arrives at structure **100** for the compound.



100

The structure **100** has been confirmed by ^{13}C NMR spectrum and the values of chemical shift of different carbons are much closer to the values reported by Koul et al.[93] (Table I). The compound **100** is thus identified as cubebin which has been earlier characterized by Crombie et al[94].

Table – I

C^{13} Chemical Shift Values of Cubebine (100)

Carbon	Cubebin δ (ppm) Reported	Cubebine δ (ppm) Found
6	39.5	39.1
7	38.7	38.7

3	53.1	53.0
4	45.1	45.7
2	103.5	103.2
5	72.7	72.5
O-CH_2O	101.1	100.7
1"	132.3	132.2
1'	133.9	133.8
2"	108.3	108.7
2'	109.3	109.0
3"	147.5	147.9
3'	147.5	147.2
4"	145.9	145.6
4'	145.9	145.7
5"	109.5	109.1
5'	109.0	109.0
6"	121.9	121.6
6'	121.6	121.2

Further confirmation for **100** is provided by mass spectrum (Fig. 36) which shows M^+ at m/z 356. The other prominent peaks arise through loss of OH group from M^+ and from the fragment ions (Scheme 39).

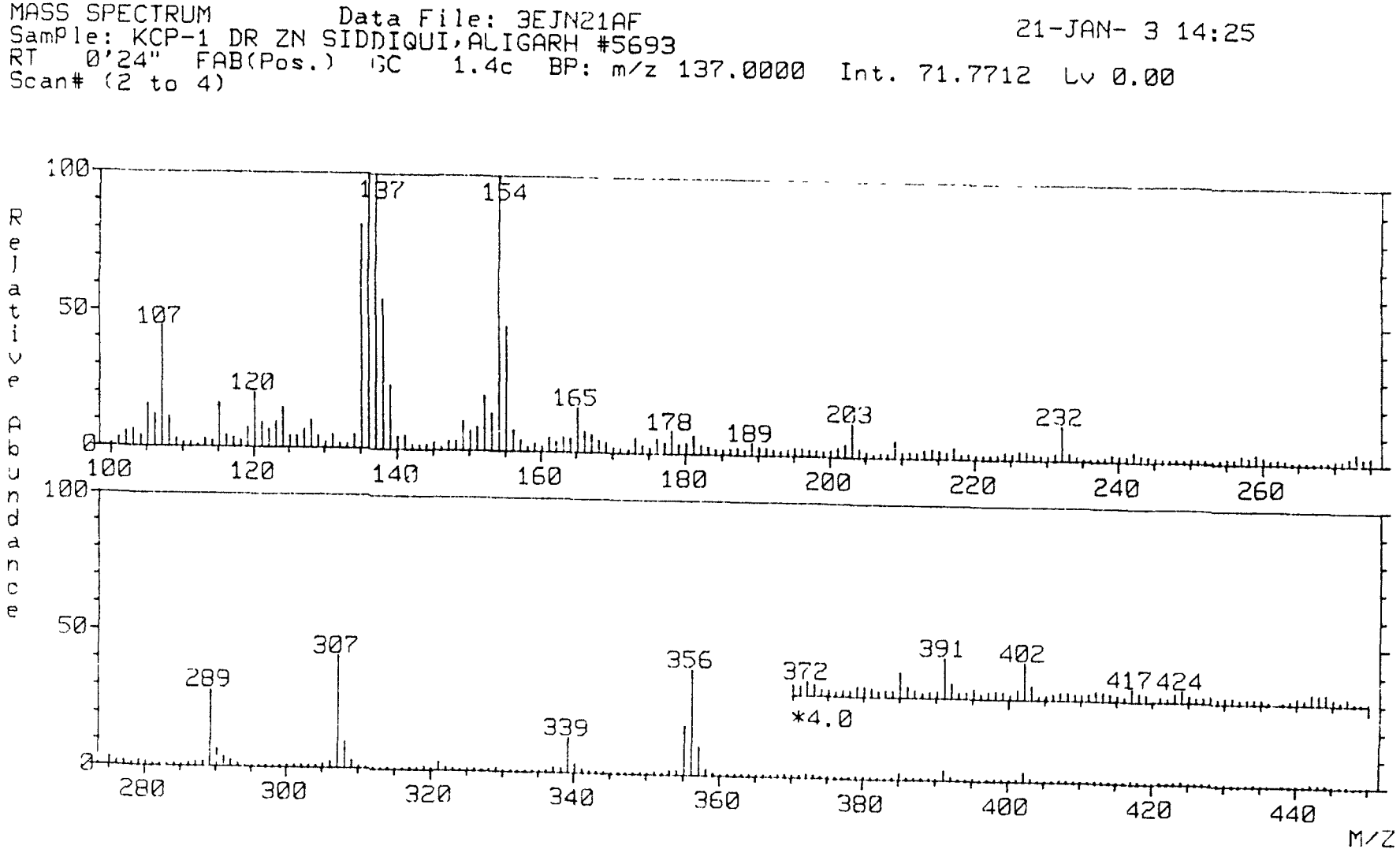
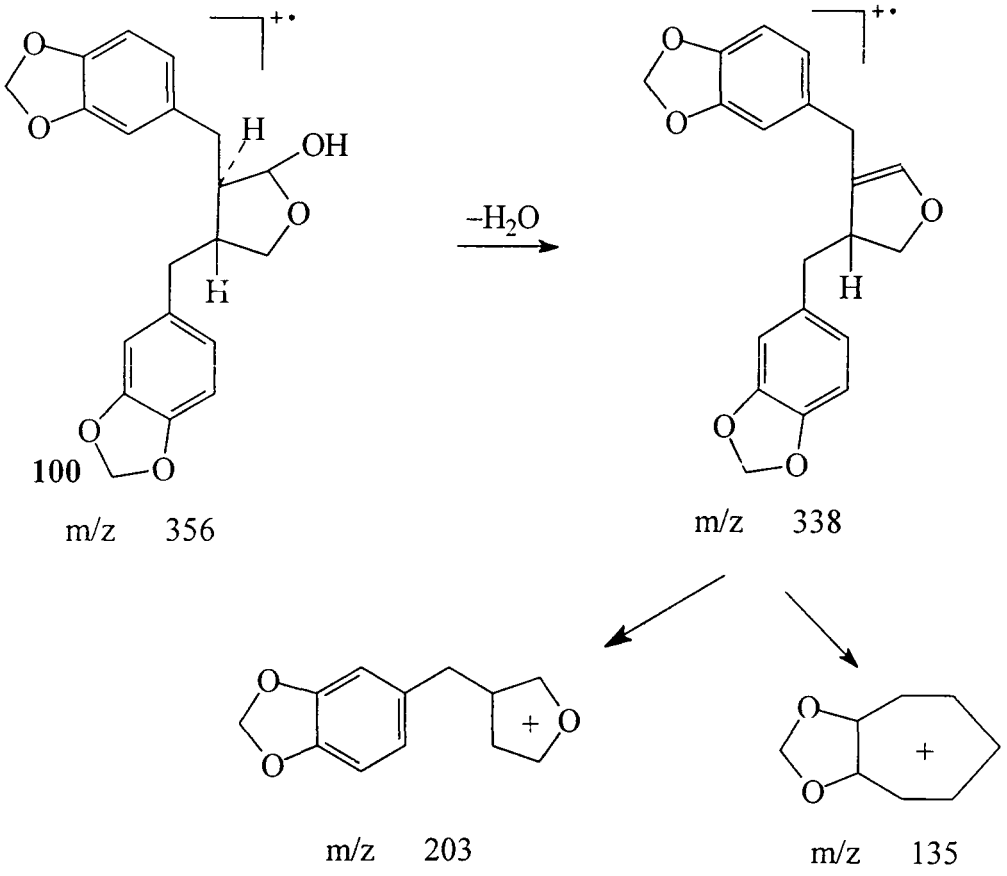

MASS SPECTRUM Data File: 3EJN21AF
Sample: KCP-1 DR ZN SIDDIQUI,ALIGARH #5693
RT 0'24" FAB(Pos.) GC 1.4c BP: m/z 137.0000 Int. 71.7712 Lv 0.00
Scan# (2 to 4)
21-JAN- 3 14:25
Relative Abundance
M/Z
137
154
107
120
165
178
189
203
232
289
307
339
356
372
391
402
417
424
*4.0

Fig. 36



Scheme 39

2.3.2 Chemical Constituents of Zanthoxylum simularis

In the context of investigation of medicinally important plants *Zanthoxylum simularis* (Rutaceae) was selected because other *Zanthoxylum* species are known to produce novel alkaloids[95-99], flavonoids[100-101] and coumarin[102]. The plant was defatted and extracted with chloroform and alcohol. The alcohol extract contained a mixture of compounds which had not responded to simple chromatographic technique available in the laboratory. Only chloroform extract of the plant could be thoroughly analysed so far.

A number of colour tests have been reported in literature for detecting particular structure features. The more commonly used colour reaction are Shinoda's test (Mg+HCl) for flavones and Liebermann Burchard reaction for terpenes

The chloroform extract was subjected to column chromatography which afforded altogether six compounds out of which only two compounds were isolated in pure form and labeled as ZS-1 and ZS-2. The remaining four compounds were obtained as impure, minor constituents, and therefore could not be subjected to further study. Both the compounds have been characterized on the basis of spectroscopic data and are discussed here.

The spectral characteristics of ZS-1 and ZS-2 indicated as a triterpene and a chromeno flavone. Thus, triterpene ZS-1 gave positive Liebermann- Burchard reaction and flavone ZS-2 formed pink colour with Mg/HCl.

ZS-1: The compound analysed for $C_{26}H_{30}O_8$ melting point 275° shows strong IR (Fig. 37) bands at 1755 and 1710 cm^{-1} indicating the presence of a lactone and a carbonyl functional groups. The NMR spectrum (Fig. 38) shows clear resonances of a α and β protons of furan ring[103]. This feature coupled with positive Ehrlisch test indicated that the compounds is a furan substituted triterpene belonging to the family of limonoids. Much work has been done on structure elucidation of

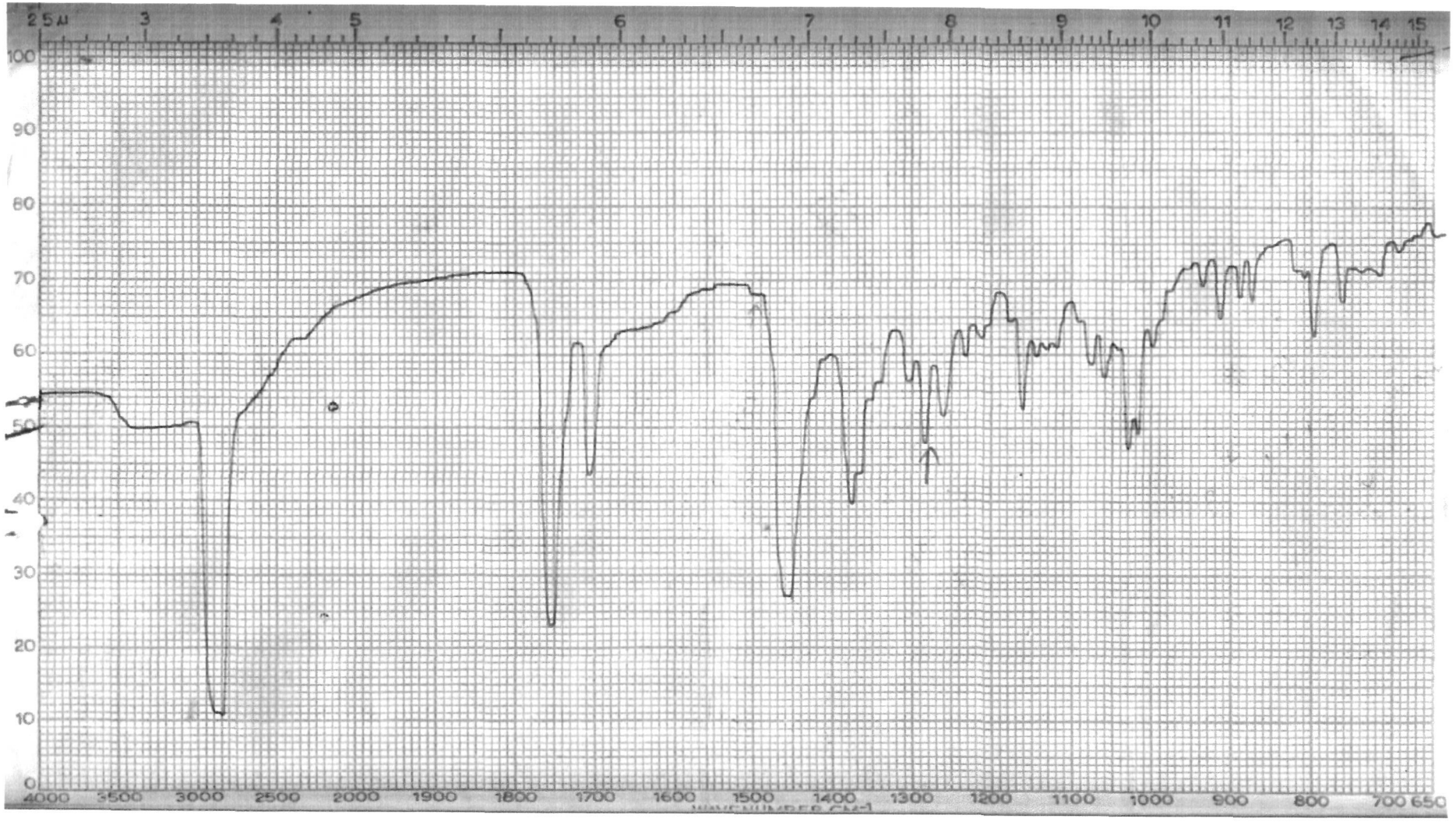


Fig. 37

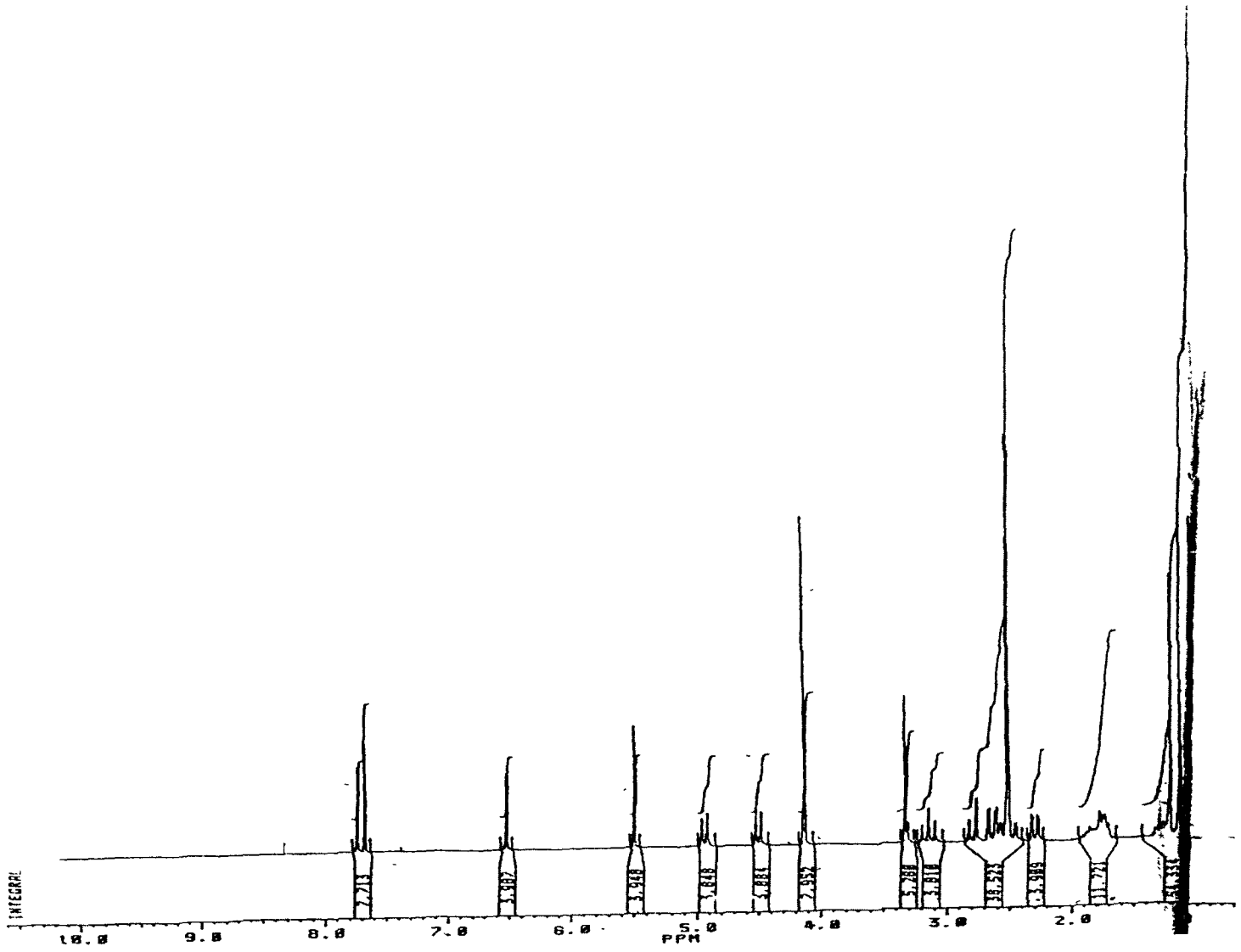


Fig. 38

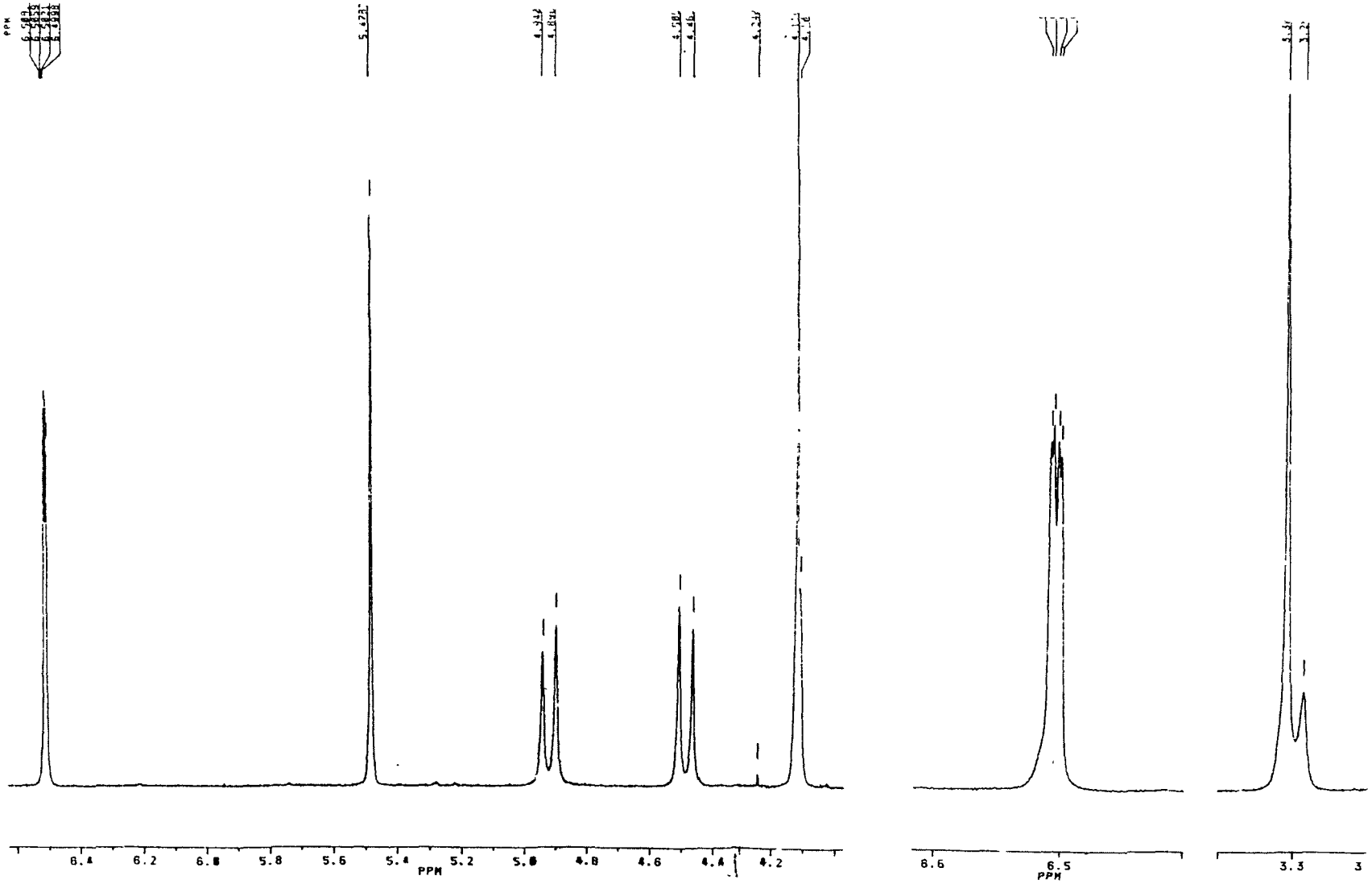
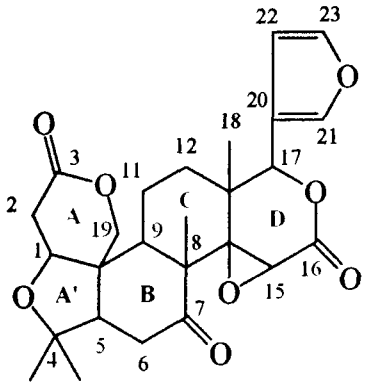


Fig. 38 (Expanded)

limonoids by D. L. Dreyer[104,105]. Therefore, assignment of chemical shifts of various protons were made in the light of findings by D. L Dreyer.

The nmr spectrum of ZS-1 shows four methyl singlets at δ 1.00 – 1.19 which suggested that the compound is related to limonin[106], **101** and not to obacunone[107] **102** which has five methyl singlets in its structure. It is C-8 methyl group which resonates in the most up field region. This is followed by resonances of gem and C-18 methyl groups. On the basis of these findings if ZS-1 has a structure similar to that of limonin then the singlets at δ 1.00,1.02,1.11 and 1.19 can be assigned to C-8, C-4 and C-18 methyl groups respectively.



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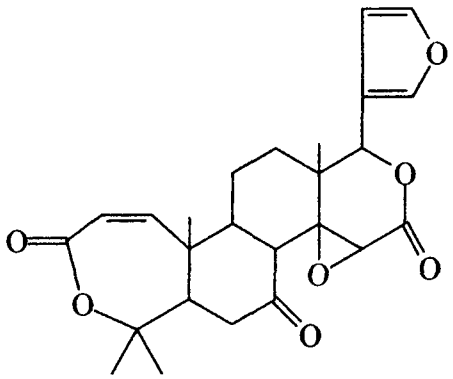
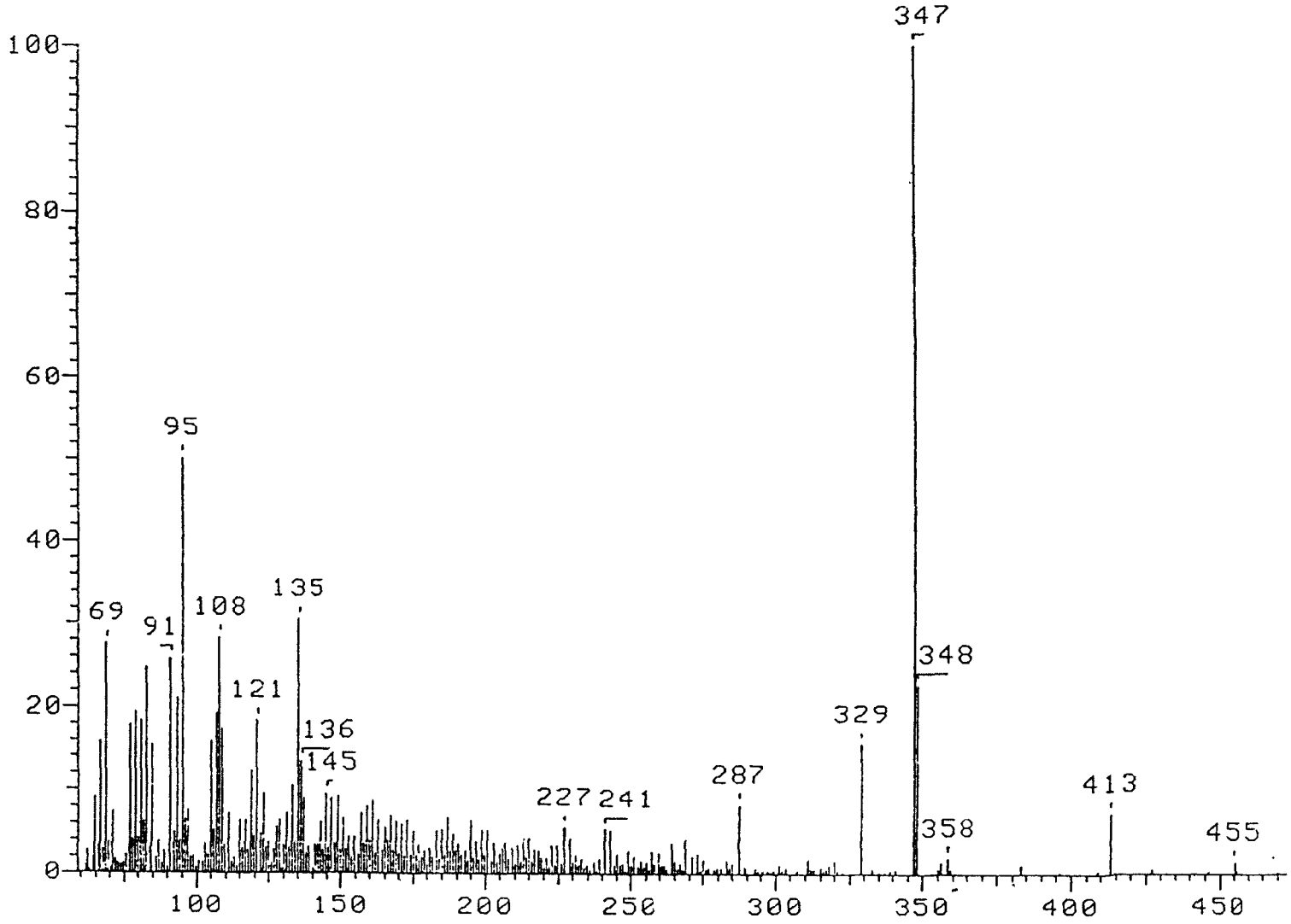


102

The aromatic region clearly shows three multiplets at δ 7.72, 7.66, 6.51 which are resonances of two α and one β protons of furan ring. A sharp singlet integrating for one proton at δ 5.47 may be assigned to furfurylic proton. Two AB doublets which appear at δ 4.90 and 4.50 (J=13

Hz) are assigned to two protons of C-19 methylene group of ring A. The sharp singlet at δ 4.12 is due to epoxy proton. Though the integration of epoxy proton is having double value, it may due to presence of some impurity, associated with the signal as shown in the expanded form of spectra (Fig. 38). The resonance at δ 3.30 can be assigned to C-5 proton as the value is quite close to that in rutavin[108] **103**. A fine triplet integrating for one proton discernible at δ 3.17 is given to H-1 proton whereas in most of the papers related to limonoids, the signal for H-1 proton is reported as unresolved multiplet. A double doublet clearly visible in the envelop situated at δ 2.42-2.80 is assigned to the one of the two protons of C-2 methylene group and resonances for other proton is lost in the envelop of protons situated at δ 2.42-2.80. Another double doublet at δ 2.31 (J=14 Hz, 3.1 Hz), integrating for one proton, appears in the spectrum as a result of coupling of C-9 proton with α and β protons of C-10 methylene protons. The value is comparable to the limonoid isolated from Atlanta monophylla[109]. Besides these, C-10 and C-11 mehtylene protons appear as multiplets situated at δ 1.68-1.87 and in the region of methyl groups and the value is closer to the limonoids isolated from *Khaya senegalensis*[110]. On the basis of these spectroscopic features, the compound is identified as limonin[106] **101.** The other structure **104** with same M^+ 470 (Fig. 39) is not possible due to biogenetic resons[111]. Another structure **105** is again not possible because


100
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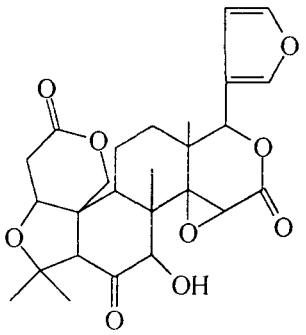
60
40
20
0
100
150
200
250
300
350
400
450
347
95
69
91
108
135
121
136
145
348
329
287
227
241
413
358
455

Fig. 39

H-1 proton in such structure usually resonates as a singlet in the region δ 6.0[112] which is not present in the NMR spectrum of **101**.



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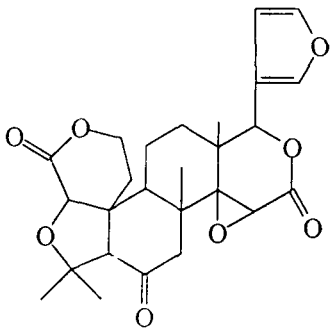
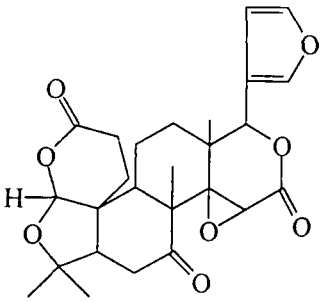


104



105

The ^{13}C NMR spectrum (Fig. 40) further provides evidences for the compound as limonin **101** and chemical shift assigned to various carbons are similar to the values reported earlier[113] (Table II).

Table 1I: ^{13}C NMR-values of (101)

Carbon	Found	Reported	Carbon	Found	Report
Me	16.66	17.1	C-13	37.5	37.6
Me	19.39	19.6	C-14	66.49	66.7
Me	21.16	21.3	C-15	53.59	53.7
Me	29.52	29.6	C-16	166.66	167.2
C-1	78.2	78.4	C-17	77.24	77.4
C-2	35.39	35.6	C-18	00	0.00
C-3	169.67	170.0	C-19	64.6	0.00
C-4	79.24	79.4	C-20	120.04	120.2
C-5	57.9	58.0	C-21	141.4	141.8
C-6	35.9	36.0	C-22	109.9	110.1
C-7	207.58	207.8	C-23	143.04	143.2
C-8	50.15	50.3			
C-9	46.3	46.2			
C-10	50.15	45.2			
C-11	17.36	17.6			
C-12	28.9	29.6			

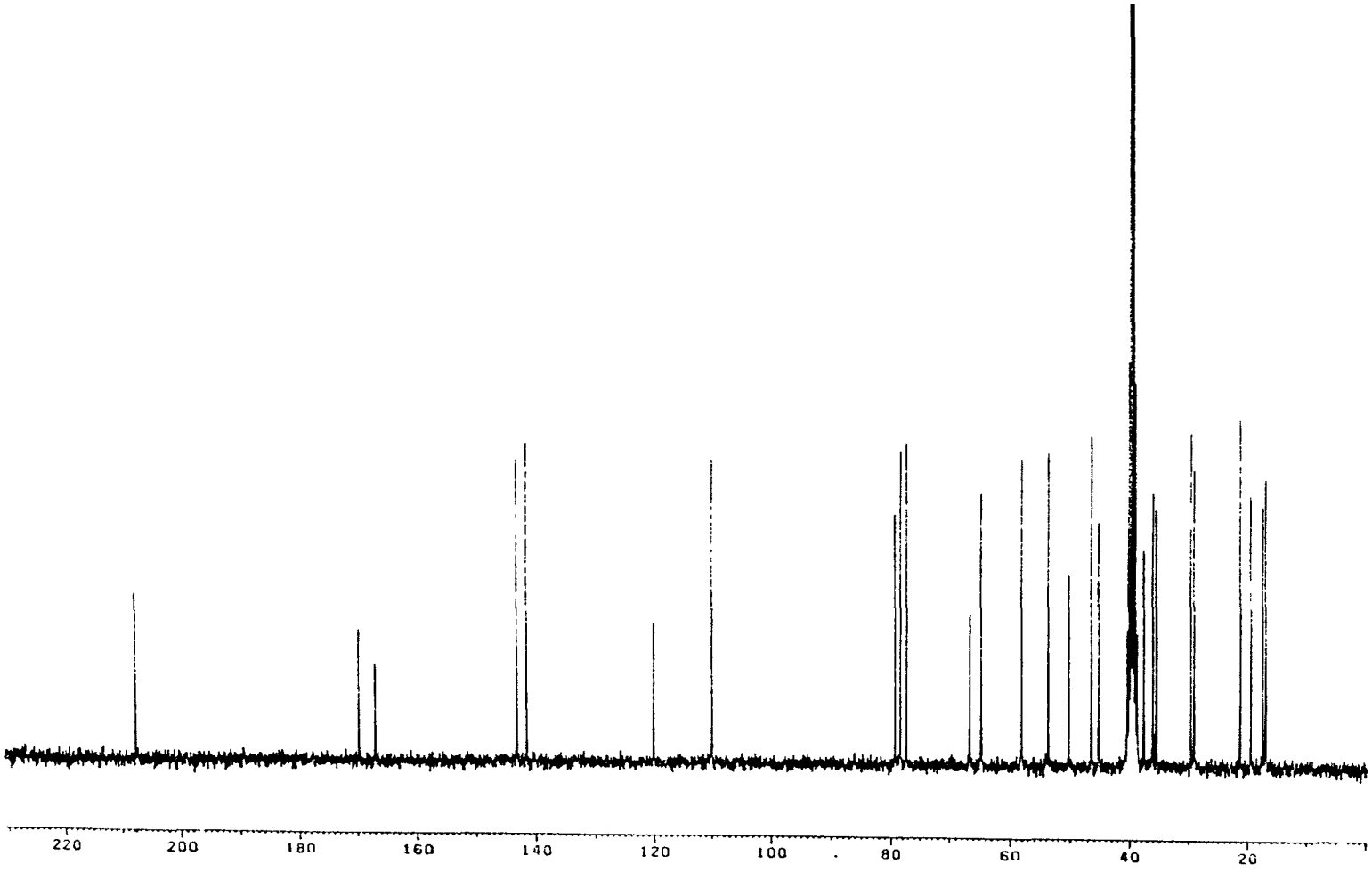
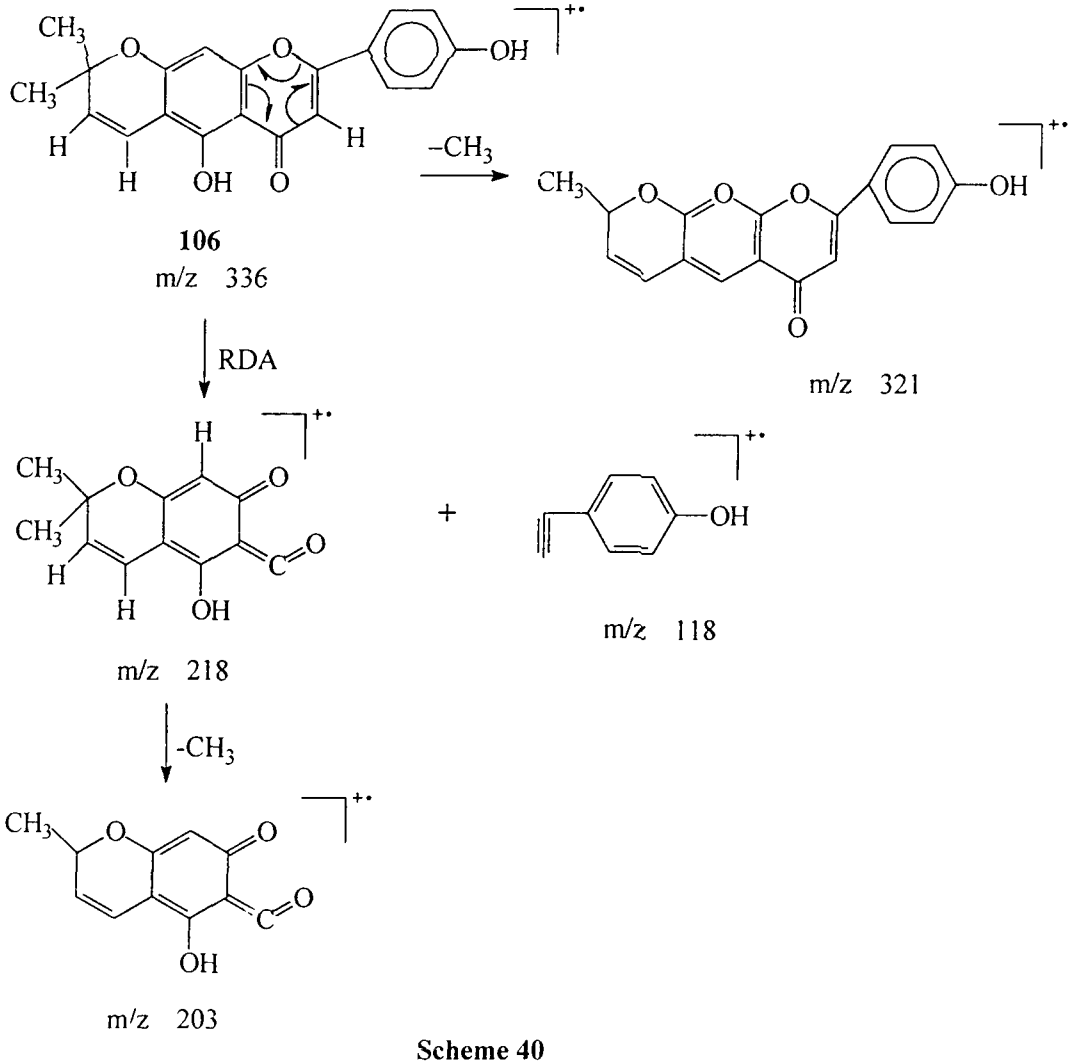


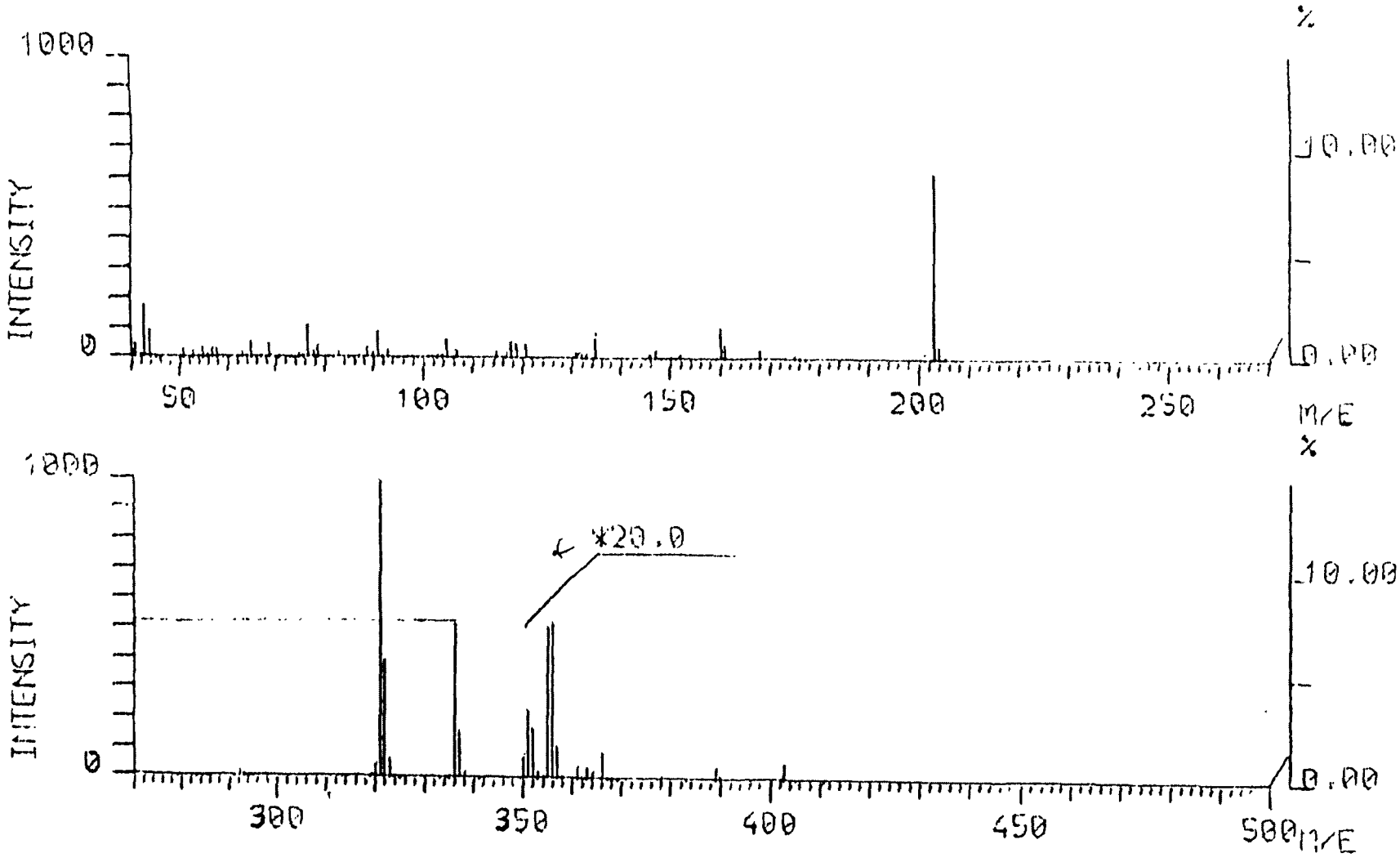
Fig. 40

ZS-2:

The compound **106** is a member of chromeno flavones and has been reported earlier by D.L. Dreyer from *Pamburus missionis* (Rutaceae)[114]. The identity of the compound m.p. 295-298 is based on spectroscopic data and shows M^+ at m/z 336 in its mass spectrum (Fig. 41). The base peak at m/z 321 is obtained due to loss of methyl group from molecular ion peak.



Scheme 40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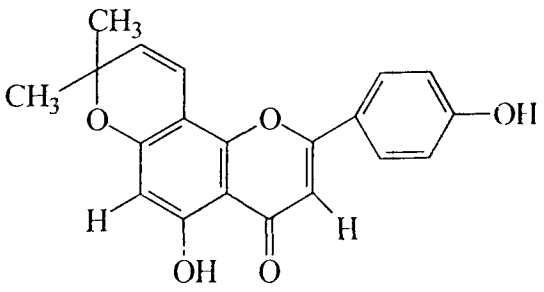
%
10.00
0.00

Fig. 41

Other peaks are obtained according to Scheme 40. The IR spectrum (Fig. 42) shows a strong and sharp band at 1660 cm^{-1} indicating the presence of chromone carbonyl group in the compound. The NMR spectrum (Fig. 43) evidences for the presence of a chromene ring by two methyl singlets at δ 1.5. One olefinic proton of chromene ring resonates as doublet (J= ~10Hz) at δ 5.6 whereas its counterpart due to other olefinic proton is not visible due to poor resolution of the spectrums Besides these, the spectrum depicts two singlets at δ 6.2 and 6.5 which may be due H-3 and H-8 protons. The other possible structure **107** for the compound which also gives two singlets is less likely because singlets for such protons usually resonate at overlapping ranges[115]. Structure **106** is, thus, more appropriate for the compound. In addition to this, the doublets at δ 7.65-7.85 and 6.9-7.1 each integrating for two protons has been assigned to 2', 6' and 3', 5' protons of ring B. Presence of C-5 hydroxyl group is established by characteristic ferric chloride test. Further confirmation for the presence of two hydroxyl groups in the form of acetate derivatives and their more exhaustive spectral data could not be made owing to paucity of material in hand.



107

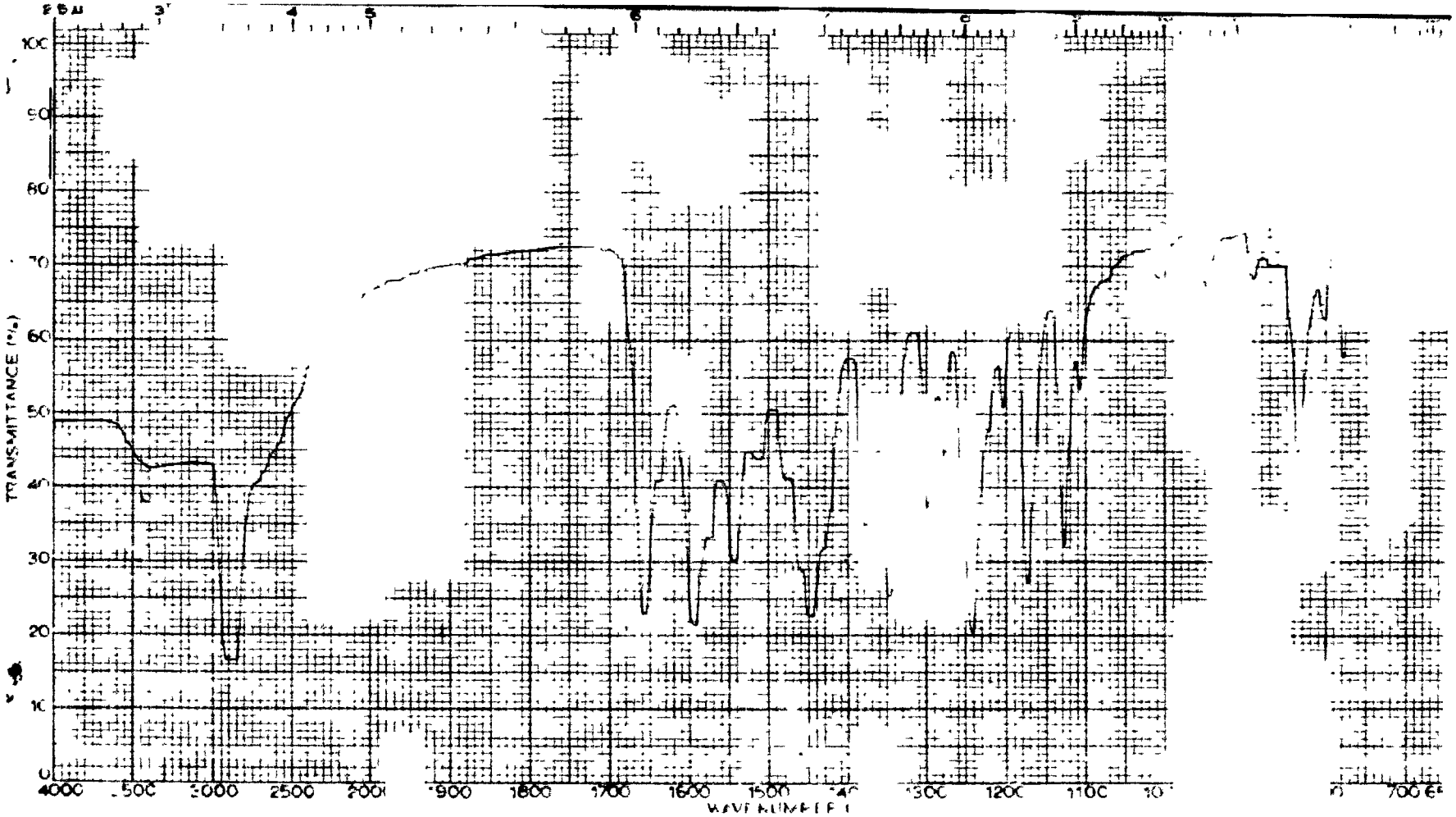

TRANSMITTANCE (%)
100
90
80
70
60
50
40
30
20
10
0
4000
3000
2500
1800
1700
1600
1500
1200
1100

Fig. 42

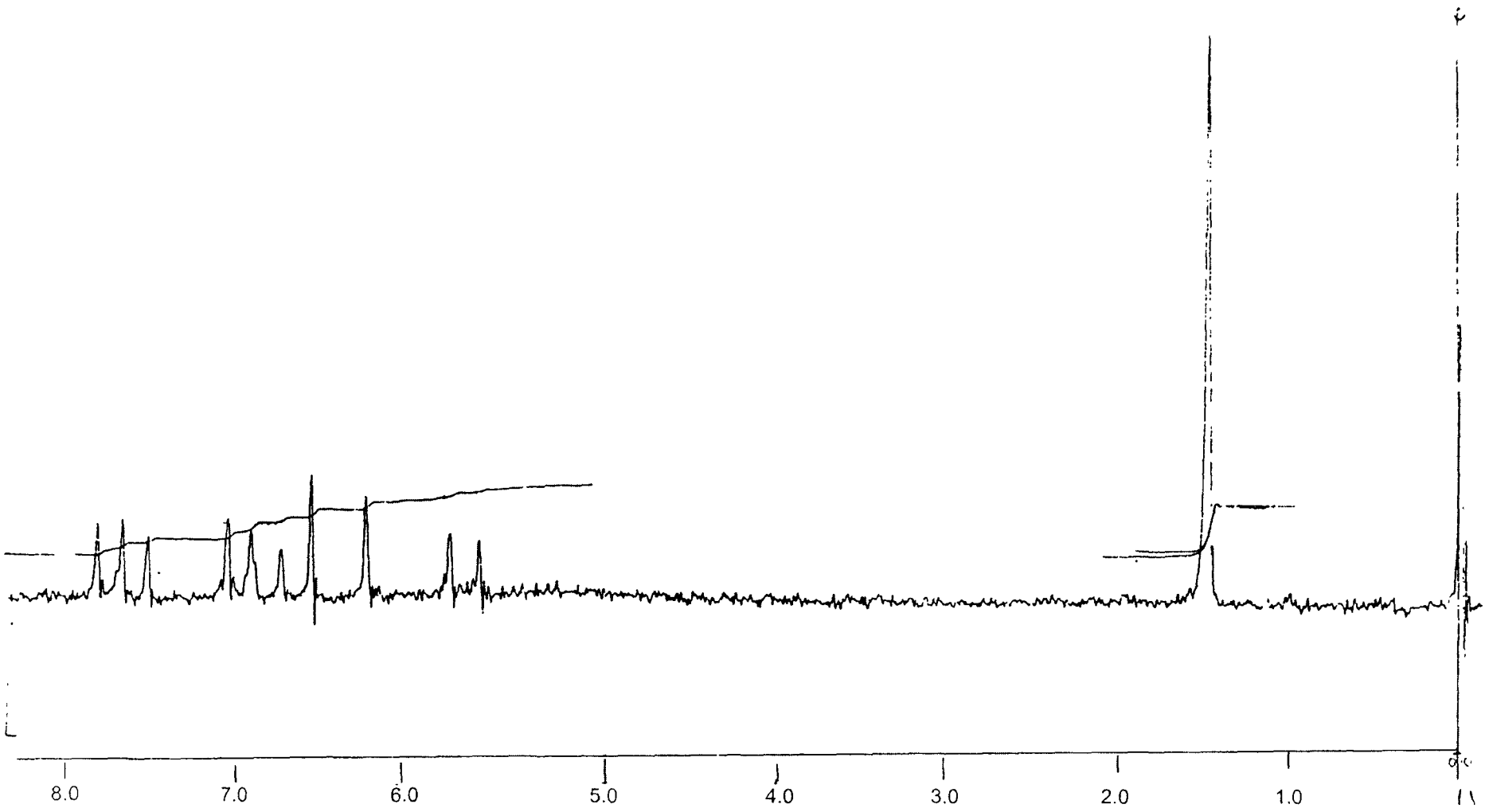


Fig. 43

CHAPTER 3

Antimicrobial Activity

3.1. Introduction

Infectious diseases are the world's leading cause of premature deaths, killing almost 50,000 people everyday. An increase in the emergence of multiple drug resistant bacteria is threatening the world population. Thus in the present scenario of antibiotic therapy there is a continuing quest for new antimicrobials drugs from other sources including plants, as they are known to produce diverse bioactive substances of chemotherapeutic value.

In this chapter we have discussed antimicrobial activity of naturally occurring and synthetic compounds.

3.2. Materials and Methods:

3.2.1 Microorganisms Used:

The test organisms used included *E. coli* U.P. 2566, (Central Drug Reseach Institute, Lucknow, India), *E. coli* K-12-J-62 (Prof. S.G.B. Amyes, University of Edinburg, UK), *Salmonella typhimurium* MTCC-98 and *Bacilus subtilis* MTCC-121 (Institute of Microbial Technology, Chandigarh, India). Clinical isolates of *Staphylococcus aureus* IOA-106, *Shigella dysenteriae* IOA-108, *Pseudomonas aeruginosa* IOA-112 and *Candida albicans* IOA-109 were kindly provided by Prof. Abida Malik, chairman, Department of Microbiology, J.N. Medical College, AMU, Aligarh, India. Dr. Mrs. Shashi Sharma, Agricultural Research Station (ICAR) Jaipur, India kindly provided the filamentous fungi like

Aspergillus niger, Fusarium solani, Fusarium chlamydosporum and *Rhizoctonia bataticola, Alternaria alternata* ITCC-4789 was obtained from Indian Type Culture Collection, IARI, New Delhi.

3.2.2 Culture Media and Inoculum:

Nutrient (N) and Sabouroud Dextrose (SD) (Hi-Media Pvt. Ltd., Mumbai, India) were used to culture the test bacteria and fungi respectively. The microbial cultures (test bacteria and *Candida albicans)* were grown at 37°C for 18 hrs. and then appropriately diluted in sterile 0.8% saline solution to obtain a cell suspension of 10^5 CFU/ml. Similarly an inoculum of viable spore/mycelial fragments (105 CFU/ml) was prepared from filamentous fungi[116].

3.2.3 Antimicorbial assays:

The disc diffusion method[117] with little modification was used. Briefly 0.1 ml of diluted inoculum (10^5 CFU/ml) of test organism was spread on nutrient agar (NA)/ Sabouraud Dextrose (SD) agar plates. Sterile paper disc impregnated with 100 μg of isolated compound in DMSO and a disc without compound was used as a negative control. The plates were incubated for 18 hrs. at 37°C for test bacteria and *Candida albicans* whereas the fungi plates were incubated for 3-5 days at 28°C. The antimicrobial activity was evaluated by measuring the zone of growth inhibition against test organism. Antibacterial antibiotics

chloramphenicol and nystatin were used in the test system as positive controls.

3.2.4 Compounds used for testing:

3.2.4.1 Cubebin (**100**):

It was isolated from shade dried seeds of *Piper cubeba* (Piperaceae). Its isolation and characterization in discussed in chapter-2

3.2.4.2 T3FC (B) (**38**), 3FCTP (**39**) 3FCTB (**40**), 3FCTNB (**42**) and 3FCTPNB (**43**):

This series of compounds was synthesized from the reaction of 3-formylchromone and 3-alkyl-4-amino-5-mercapto 1,2,4-triazole and their characterization is described in chapter 1.

3.2.4.3 4HCTUD (**48**), 3HNaL1 (**62**), 3HNaL (**68**), 3HNaD (**78**) and 3HNaPz (**81**):

These compounds were synthesized from 4-hydroxycoumarine and 2-amino-3-formylchromone as starting materials which are converted to products 4HCTUD **48**, 3HNaL1 **62**, 3HNaL **68**, 3HNaD **78** And 3HNaPz **81** by conducting the reaction with 2-ureidomethylene-cyclohexane-1,3-dione, triacetic acid lactone, 5,5-dimethylcyclohexane-1,3-dione and 3-methyl-1-phenyl-5-pyrazolone. Their synthesis and characterization are discussed in chapter 1.

3.3. Results and Discussion:

3.3.1 Antimicrobial activity of Cubebin (100) from Piper cubeba:

Piper cubeba (Piperaceae) is a well known Indian medicinal plant used traditionally against a number of ailments such as throat infection, laryngitis, asthma, bronchitis, fever, pain in abdomen and genito-urinary disorders like crystitis, gonorrhoea, gleet and leucorrhoea etc[118,119].

In view of the medicinal properties reported for the plant *P. cubeba* it was though worth while to isolate and explore biological activities of active constituents from seeds of *P. cubeba*. The present work, thus aims to evaluate the antibacterial and antifungal activities of cubebin **100** and are discussed below:

Isolated lignan (cubebin) from *Piper cubeba* (Piperaceae) was tested against a variety of organisms using dimethyl sulfoxide (DMSO) as a solvent. Table III represents the summary of the antimicrobial activity of cubebin with respect to each of the test organism at a concentration of 100 µg/ disc. The antimicrobial activity was found against all test organism with zone inhibition size ranging from 13-16 mm against test bacteria and 13-18 mm against all the fungi tested.

The compound exhibited broad-spectrum antibacterial activity against all the test bacteria including Gram –ve (*E. coli, Salmonella typhimurium, Shigella dysenteriae, Pseudomonas aeruginosa*), and Gram +ve (*Bacillus subtilis* and *Staphylococcus aureus*) with zone of

growth inhibition from 13-16 mm. Similary the compound also showed braod-spectrum activity against all the test fungi , *(Aspergillus niger Rhizoctonia bataticola, Alternaria alternata* and *Fusarium chlamydosporum)* including *Candida albicans* (Table III).

Table III

Organism	Test organism	Inhibition zone size in mm	Inhibition zone size by antibiotic in mm
Gram +ve bacteria	*Staphylococcus aureus*	13	18
	Bacillus subtilis		22
Gram –ve Bacteria	*E. coli UP-2566*	16	21
	E. coli-K-12-J-62	15	19
	Salmonella typhimurium MTCC 98	13	19
	Pseudomonas aeruginosa	13	20
	Shigella dysenteriae	13	21
Yeast	*Candida albicans*	14	17
Filamentous fungi	*Rhizoctonia bataticola*	14	13
	Fusarium solani	18	18
	Aspergillus niger	17	18
	Alternaria alternata	13	17

* Antibiotic control: Chloramphenicol (30 µg/ disc) for bacteria, Nystatin (100 units/ disc) for fungi and yeast.

3.3.2 Antimicrobial activity of compounds 38, 39, 40, 42 and 43:

1.2.4-Triazole and its derivatives have been used extensively in medicine and industry. The fused ring systems consisting of 1.2.4-

triazole moiety possess varied range of biological activities such as antibacterial, antifungal, analgesic and diuretic[120,121], antihypertensive[122], antituberculosis[123], antiinflammatory[124], herbicidal[125] and antiallergic agents[126]. Seeing diverse biological activities of compounds possessing 1,2,4-triazole ring system we have explored antimicrobial activity of compounds having both chromone and 1,2,4-triazole moieties. It is pertinent to mention that chromone derivatives possess analgesic, anti-inflammatory[127] and antiallergic activities[128], antiplatellets and cytotoxic activities[129,130].

All compounds were evaluated for their antibacterial and antifungal activities using DMSO as solvent. Interestingly only compounds **38-40** exhibited pronounced broad-spectrum activity against both bacteria and fungi. The compound **40** showed highest activity against *Staphylococcus aureus* (Gram positive) as well as *Salmonella typhimurium* (Gram negative) as compared to **38** and **39**. However, **40** was not active against *Pseudomonas aeruginosa*. Similarly **38** showed relatively less activity to all test bacteria except *Salmonella typhi*. The compound **39** showed activity against all test bacteria. The zone inhibition size of these compounds is comparable with zone inhibition obtained by broad-spectrum antibiotic, chloramphenicol (Table IV). Similarly, compound **40** demonstrated highest activity against *C. albicans* and the activity is comparable with antifungal drug, nystatin. More interestingly the compounds **40** and **39** showed strong antifungal activity against filamentous fungi (*M. phaseolina, A. niger, F.*

solani and *H. oryzae*) as compared to antifungal drug. However, the antifungal activity of **38** is at par with nystatin against *A. niger, F. solani* and *H. oryzae*. In general the antimicrobial activity was in order of **40** > **39** > **38** (Table IV).

Table IV

Antibacterial and antifungal activities of 38-40.

Organism	Test organisms	Inhibition zone diameter (mm) of test compound			
		38 (50µg/ disc)	**39** (50µg/ disc)	**40** (50µg/ disc)	Antibiotic control* (30µg/ml)
Gram +ve Bacteria	*Staphylococcus aureus*	12	15	22	18
	Bacillus subtilis	08	14	15	22
Gram –ve Bacteria	*E. coli UP–2566*	09	12	12	19
	E. coli K12–J62	12	11	11	21
	Salmonella typhimurium MTCC98	10	12	16	19
	Salmonella typhi	--	14	14	20
	Pseudomonas aeruginosa	13	13	-	21
Yeast	*Candida albicans*	11	11	15	17
Filamentous fungi	*Macrophomina phaseolina*	31	37	37	21
	Aspergillus niger	14	31	29	18
	Fusarium solani	15	25	30	16
	Helminthosporum oryzae	15	22	21	16
	Fusarium chlomydosporum	-	-	19	

* Antibiotic control: Chloramphenicol (30µg/ disc) for bacteria, Nystatin (100units/disc) for fungi and yeast.

3.3.3 Antibacterial activity of 62 and 68:

Triacetic acid lactone[131], TAL, is a polyketide as suggested by J.N. Collie[132] for polyketo methylene compounds $CH_3(-CO-CH_2-)_n$ and is involved in the biosynthesis of natural products. Recently it has been reported that type III polyketide synthases (PKS III) which are small homodimeric proteins catalyse the biosynthesis of aromatic polyketide in plants and bacteria[133,134]. A number of type III PKS are present in plants other than chalcone synthase which is used for catalyzing naringenin chalcone, a precursor of flavonoids and isoflavonoids[135,136]. This includes 2-pyrone synthases (PS) which gives TAL (4-hydroxy-6-methyl-2-pyrone). PS forms TAL by lactonization of a tirketide intermediate[137]. TAL is, therefore, precursor of a phytoalexin in plant Gerbara hybrid[138,139]. Thus, polyketides form a very large and diverse group of natural products having important physiological effects.

Besides these, compounds possessing varied pharmacological properties have also been synthesized from TAL. This includes heterocyclic ketone which induces the production of thrombocytes, leukocytes erythrocytes and are, thus effective in cancer chemotherapy[140,141]. Other derivatives of TAL are used as anticanvulsants and antiepileptics[142] whereas tricyclic and tetracylic pyrones have been used as anticancer agents [143,144].

In view of wide range of pharmacological activities of compounds from TAL we have made an attempt to explore the antibacterial and

antifungal activities of compounds synthesized from the reaction of triacetic acid lactone and 2-amino-3-formylchromone.

The compounds **62** and **68** showed antibacterial activity against gram +ve bacteria and no activity against gram –ve bacteria (Table V). The activity was determined on the basis of growth inhibition zone which ranged from 9-13 mm against *Bacillus subtilis* and *Staphylococcus auerus*. Though both **62** and **68** showed moderate activity against *S. aureus* and *B. subitilis*, the compound **68** showed higher antibacterial activity as compared to **62**.

Similarly both the compounds exhibited broad spectrum antifungal activity against test fungi including *Candida albicans*. Interestingly both **62** and **68** showed very promising activity against *Candida albicans* as compared to antifungal drug nystatin against which *Candida albicans* exhibited resistance. The compound **62** exhibited fair activity against *H. oryzae* and *M. phasiolina* where as **68** showed good activity against *Aspergillus niger*, *Helminthus oryzae* and *Macrophomina phaseolina* (Table V). It is interesting to mention here that both the compounds did not show any activity against *F. chlamydosporum* and *F. solani* (both fungi belonging to the same genera). The activity was found between 11-13 mm. against test fungi.

Table V: Antibacterial and antifungal activities of (62) and (68).

Organisms	S.No.	Test organisms	Inhibition zone diameter (mm) of test compounds		
			(50 µg/disc) **68**	(50 µg/disc) **62**	Antibiotic control (30 µg/ml)
Gram +ve Bacteria	1	*Bacillus subtilis*	13	10	38
	2	*Staphylococcus aureus*	9	9	33
Gram –ve	3	*Salmonella typhi*	-	-	16
	4	*Pseudomonas aeruginosa*	-	-	26
	5	*Escherichia coli (UP2566)*	-	-	30
	6	*Salmonella typhimurium (MTCC98)*	-	-	35
Yeast	7	*Candida albicans*	11	12	-
Filamentous fungi	8	*Fusarium chlamydosporum*	-	-	19
	9	*Aspergillus niger*	13	-	18
	10	*Fusarium solani*	-	-	18
	11	*Helminthosporum oryzae*	11	10	16
	12	*Macrophomina phaseolina*	12	10	21

3.3.4 Antimicrobial activity of 48, 78 and 81:

The present work which is also the concluding part of the thesis, is devoted to the biological evaluation of compounds synthesized from the reaction of 4-hydroxycoumarin and ureidomethylenecyclohexane-1,3-dione, 2-amino-3-formylchromone and dimedone, 2-amino-3-formylchromone and 3-methyl-1-phenyl-5-pyrazolone. It is important to

mention here that different azaxanthones[145] and coumarin derivatives[146,147] of pharmacological interest have been syntehsized employing 2-amino-3-formylchromone and 4-hydroxycoumarin as starting materials.

The compounds showed antibacterical activity against gram +ve bacteria only. The activity was determined on the basis of size of growth inhibition of test organism around the disc and was found to range from 8-13 mm against *Bacillus subtilis* and *Staphylococcus aureus*. In this series of compounds **78** showed highest activity followed by **48** and **81** (Table VI) against *Staphylococcus aureus*. Similarly the relative antibacterial activity was in the order **48>81>78** against *Bacillus subtilis*. In comparison with control (antibiotic) the activity exhibited by these compound are comparable.

All compounds, similarly were screened for antifungal activity against test fungi (*Aspergillus niger, Fusarium chlamydosporum, Fusarium solani, Helminthosporum oryzae and Macrophomina phaseolina*) including *Candida albicans*. All compounds exhibited very good activity against *Candida albicans* as compared to antifungial drug nystatin against which the fungus was resistant. The antifungal activity was in order **48>81>78** agaisnt *Candida albicans*. The compound **78** showed quite good activity against all test fungi (Table VI) as compared to nystatin. The compound **78** exhibited fair activity against three types

of fungi, namely, *Aspergillus niger, Helminthosporum oryzae and Macrophomina phaseolina* and no activity against *Fusarium chlamydosporum* and *Fusarium solani* whereas compound **81** showed good activity against two fungi of the same genera i.e. *Fusarium chlamydosporum and Fusarium solani* whereas no activity was shown against *Aspergillus niger, Helminthoporum oryzae and Macrophomina phaseolina.* The activity was comparable to the antifungal drug nystatin.

Table VI: Antibacterial and antifungal activities of (48), (78) and (81).

Organisms	S.No.	Test organisms	Inhibition zone diameter (mm) of test compounds			
			(50 µg/disc) **48**	(50 µg/disc) **78**	(50 µg/disc) **81**	Antibiotic control (30 µg/ml)
Gram +ve Bacteria	1	*Bacillus subtilis*	11	8	9	38
	2	*Staphylococcus aureus*	12	13	12	33
Gram –ve	3	*Salmonella typhi*	-	-	-	16
	4	*Pseudomonas aeruginosa*	-	-	-	26
	5	*Escherichia coli (UP2566)*	-	-	-	30
	6	*Salmonella typhimurium (MTCC98)*	-	-	-	55
Yeast	7	*Candida albicans*	14	09	11	-
Filamentous fungi	8	*Fusarium chlamydosporum*	13	-	14	19
	9	*Aspergillus niger*	10	11	-	18
	10	*Fusarium solani*	12	-	14	18
	11	*Helminthosporum oryzae*	10	11	-	16
	12	*Macrophomina phaseolina*	12	10	-	21

CHAPTER 4

Experimental

General:

The melting points were taken in open capillaries and are uncorrected. The Infra-red spectra were recorded on Perkin Elmer RXI spectrometer using KBr. Ultra-voilet spectra in 95% methanol were measured on USB 2000 Ocean-Optics spectrophotometer and wave lengths, λ_{max} were expressed in nm. The ^1H NMR spectra were measured on Bruker DRX-300 MHz in deuteriochloroform or hexamethyldeuteriodi-methylsulfoxide with TMS as internal standard. The mass spectra were obtained in FAB mode on Jeol Sx 102 using Argon/Xenon as the FAB gas. m-Nitrobenzyl alcohol was used as the matrix. Peaks at m/z 136, 137, 154, 289, 307 which appears in the mass spectrum are due to matrix. The purity of the compounds were checked by TLC on glass plates coated with silica Gel G (Merk-Germany) using benzene and ethyl acetate as mobile phase and visualized by iodine vapour.

All the solvents and chemicals used were AR grade. 2-Hydroxyacetophenone, dehydroacetic acid, phosphorousoxychloride, 4-hydroxycoumarin, N,N-dimethyl formamide and hydroxyl ammonium sulphate were obtained from E. Merck (Germany).

3-Formylchromone[8], 4-amino-3alkyl-5-mercapto-1,2,4-triazoles[43], 2-ureidomethyl enecyclohexane-1,3-dione[46], 2-amino-3-formyl-

chromone[51] and triacetic acid lactone[131] were prepared according to published procedures.

The reaction of 3-formylchromone with 4-amino-3-methyl-5-mercapto-1,2,4-triazole.

Formation of 3-(3-methyl-5-mercapto-1,2,4-triazolyliminomethyl) chromone (38)

3-Formyl chromone **1** (1 gm, 5.75 mmole) was dissolved in dry benzene (10ml) and 4-amino-3-methyl-5-mercapto-1,2,4-triazole (0.747 gm, 5.75 mmole) added to it followed by p-toluene sulfonic acid (few crystals). The reaction mixture was refluxed on water bath for 16 hours, concentrated and allowed to stand at room temperature when light yellow solid precipitated out. It was filtered, washed with alcohol, dried and recrystallized from chloroform to give 3-(3-methyl-5-mercapto-1,2,4-triazolyliminomethyl) chromones, **38**.

yield : 1.07gm (65%)

M.P. : 210-212°C

Spectral Data:

UV(MeOH)λ_{max}: 247.25, 298.33, 338.14 nm.

IR (KBr) λ_{max}: 3424, 3060, 2931, 2752, 2373, 1649, 1591, 1494, 1456, 1405, 1284, 1226, 1094, 981, 832, 752 cm^{-1}.

^1H NMR (300 MHz, DMSO-d_{16}):

δ 2.37 s, 3H (CH_3); 7.57-7.93 m, 3H (Ar-H); 8.17d, 1H, 9 Hz (C-5); 9.13 s, 1H (C-2); 10.32 s, 1H (-CH=N-).

MS (rel. int.): m/z 286 (M^+ 84%), 172 (52), 167 (4), 165 (8), 139 (14), 138 (29), 137 (47), 136 (80), 120 (15), 114 (12).

The reaction of 3-formylchromone with 4-amino-3-ethyl-5-mercapto-1,2,4-triazole

Formation of 3-(3-ethyl-5-mercapto-1,2,4-triazolyliminomethyl) chromone (39)

A mixture of 3-formyl chromone **(1)** (1.0 gm, 5.75 mmole) and 4-amino-3-ethyl-5-mercapto-1,2,4-triazole (0.827 gm, 5.74 mmole) was dissolved in dry benzene (10 ml) and refluxed for 18 hour in the presence of catalytic quantity of p-toluene sulfonic acid. The reaction mixture was cooled at room temperature to afford light yellow solid. It was filtered, washed with benzene and recrystallized from chloroform to give 3-(3-ethyl-5-mercapto-1,2,4-triazolyliminomethyl) chromones **39**.

Yield : 1.07 gm (62%)

M.P. : 240°C

Spectral Data:

UV (MeOH) λ_{max}: 251.75, 297.22, 339.61 nm.

IR (KBr) ν_{max}: 3451, 3098, 2932, 2365, 1654, 1568. 1462, 1231, 1145, and 761 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$):

δ 1.33 t, 3H (CH_3); 2.82 q, 2H (CH_2); 7.48-7.78 m, 3H (Ar-H); 8.33 d, 1H, J=9Hz (C-5); 8.70 s, 1H (C-2); 10.37 s, 1H (-CH=N-); 10.46 br s, 1H (NH).

MS (rel. int): m/z 300 (M^+ 100%), 172 (83), 165 (4), 138 (4), 123 (2), 120 (8), 114 (4).

The reaction of 3-formylchromone with 4-amino-5-mercapto-3-propyl-1,2,4-triazole:

Formation of 3-(5-mercapto-3-propyl-1,2,4-triazolylinomethyl) chromone (40):

3-Formylchromone (1.0 gm, 5.75 mmole) was dissolved in dry benzene (10 ml) and (0.908 gm, 5.74 mmol) 4-amino-5-mercapto-3-propyl-1,2,4-triazole, added to it. The reaction mixture was refluxed on water bath for 20 hours in the presence of catalytic amount of p-toluene sulfonic acid. It was concentrated, allowed to stand at room temperature when light yellow solid precipitated out. It was filtered, washed with a mixture of benzene and petrol, dried and recrystallized from chloroform to give 3-(5-mercapto-3-propyl-1,2,4-triazolyliminomethyl) chromone **40**.

Yield : 1.105 gm (61%)

M.P.: 225°C.

Spectral Data:

UV (meOH) λ_{max}: 251.38,296.11,350 nm.

IR(KBr) ν_{max}: 3088, 2948, 2773, 1646, 1563, 1464, 1282, 1236 and 764 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$):

δ 1.041 t, 3H (CH_3); 1.82 m, 2H (CH_2); 2.78 t, 2H (CH_2); 7.48-7.79m, 3H (Ar-H); 8.30-8.33dd, 1H, J=7.8 Hz, 0.9Hz, (C-5); 8.71 s, 1H (C-2); 10.35 s, 1H (-CH=N-); 11.22 br s, 1H (NH).

MS (rel. int.): m/z 314 (M^+ 84%), 172 (100), 165 (12), 138 (18), 123 (9), 120 (12), 114 (12).

The reaction of 3-(3-alkyl-5-mercapto-1,2,4-triazolyliminomethyl) chromone 38-40

Formation of 2-(4-oxo-4H-[1] benzopyran-3-yl) [1] benzopyrano-[3,2-e] pyrimidin-5(5H)-one, (42) and 1-(4-oxo-4H-[1] benzopyran-3-yl) [1] benzopyrano-[3,2-d] pyridazin-5(5H)-one (43)

The compounds **38-40** (1.0 gm, 3.49 mmole) were taken in nitrobenzene (10 ml) and refluxed on an oil bath for 4 hours. The reaction mixture was adsorbed on a column of silica Gel and eluted with benzene. Appropriate fractions were combined, evaporated and crystallized from benzene petroleum ether to afford compound 2-(4-oxo-4H-[1] benzopyran-3-yl) [1] benzopyrano-[3,2-e] pyrimidin-5(5H)-one, **42**[151] as a yellow crystals.

Yield : 0.72 gm (60%),

M.P. : 220-222°C

Spectral Data:

UV (MeOH) λ_{max}: 244.25, 291.66, 402.10 nm.

IR (KBr) ν_{max}: 1652, 1602, 1531, 1402 cm^{-1}.

1H NMR (300 MHz, DMSO d_6):

δ 7.57-8.04 m, 6H (Ar-H); 8.24 d, 2H (H_C); 9.14 s, 1H (H_a); 9.58 s, 1H (H_b).

MS (rel. int.): m/z 342 (M^+ 90%), 315 (2), 284 (6), 267 (2), 223 (4), 190 (5), 172 (6), 144 (2), 120 (2), 107 (2).

Further elution of the column with benzene and ethylacetate (90:10, v/v) gave 1-(4-oxo-4H-[1] benzopyran-3-yi) [1] benzopyrano-[3,2-d] pyridazin-5(5H)-one **43**. It was recrystallized from benzene petroleum mixture as yellow crystal.

Yield : 0.92 gm (40%),

M.P. : 240°C.

Spectral Data:

UV (MeOH) λ_{max}: 241.25, 262.98, 288.69 nm.

IR (KBr) ν_{max}: 1672, 1616, 1579, 1454, 1392, 1332, 791 and 717 cm^{-1}:

1H NMR (300 MHz, $CDCl_3$):

δ 7.26-7.63 m, 6H (Ar-H); 7.65,dd, 1H(H_c); 8.24 dd, 1H (H_C); 9.80 s, 1H (H_a); 10.30 s, (1H H_b).

MS (rel, Int): m/z 342 (M^+ 20%), 315 (6), 313 (6), 290 (9), 286 (6), 222 (6), 172 (3), 120 (17), 116 (3).

The reaction of 3-formylchromone with dimedone

Formation of 2-(4-oxo-4H-1-benzopyran-3-yl-methylidene)-5-, 5-dimetyl cyclohexane-1, 3-dione (45):

3-Formylchromone (1 g, 5.75 mmol) was dissolved in absolute alcohol (20 ml) and dimedone (0.805 g, 5.75 mmol), fused sodium acetate (0.471 g, 5.75 mmol) were added to it. The reaction mixture was refluxed on water bath for 5 hours, concentrated and allowed to stand at room temperature. The white solid 2-(4-oxo-4H-1-benzopyran-3-yl methylidene)-5, 5-dimethyl cyclohexane-1, 3-dione **45** crystallized out from the reaction mixture, was filtered, washed with alcohol, dried and recrystallized from chloroform.

M.P. = 220-222°C

Yield = 1.45 (85%)

Spectral Data:

UV (MeOH) λ_{max}: 253.25, 292.03 nm

IR (KBr): 3421, 1630, 1569, 1467, 1408, 1349, 1323, 1148 and 764 cm^{-1}

^1H NMR ($CDCl_3$): δ 0.978 6H, s, (CH_3), 2.23 2H, s (CH_2), 2.55 2H, s (CH_2), 7.32-7.57 4H, m (ArH+CH), 7.65 1H, d, J=7.8 Hz (H-6), 8.12 (1H, d, J=7.8 Hz, H-5), 8.32 1H, s (H-2).

MS (rel. Int): m/z 295 (M^+-1, 25%), 294 (92), 278 (8), 258 (6), 232 (14), 229 (14), 176 (14), 165 (11), 154 (100), 121 (17), 107 (28) and 105 (11).

The reaction of 4-hydroxycoumarin with 2-ureidomethylene cyclohexane-1, 3-dione

Formation of 7-(4-hydroxycoumarin-3-yl) 10,10-dimethyl-8-oxo-8, 9,10,11-tetrahydro pyrano (3,2-c) coumarin (48).

To a solution of 4- hydroxycoumarin (0.764g, 4.71 mmol) and 2-ureidomethylenecyclohexane-1, 3-dione (1.0g, 4.71 mmol) in glacial acetic acid (30 ml), was added fused sodium acetate (1.0g). The reaction mixture was refluxed for six hours. It was then cooled at room temperature and added to water. The solid as obtained, was filtered, washed with water, dried and crystallized from ethanol to give 7-(4-hydroxycoumarin-3-yl) 10,10-dimethyl-8-oxo-8, 9,10,11-tetrahydro pyrano (3,2-c) coumarin **48** as white shining crystals.

M.P. 235-240

Yield 1.2g,56%.

Spectral Data:

UV (MeOH) λ_{max}: 261.11, 309.43.

IR (KBr)ν_{max}: 3432, 1722, 1617, 1374, 1326, 1193, 1034, 755 cm^{-1}

^1H NMR (DMSO):δ 0.98 s, 3H (CH_3), 1.10 s, 3H (CH_3) 2.16 d, 1H, J_{ab}=15.9 Hz (H_a), 2.39 d, 1H, J_{ba} = 15.9 Hz (H_b), 2.64 d, 1H, J_{cd}=17.7 Hz (H_c), 2.79 d, 1H, J_{dc}=17.7

Hz (H_d), 5.26 s, 1H, 7.27-7.73 m, 6H (Ar-H), 7.93 d, 1H, J=7.8 (H-1), 8.01 d, 1H, J=8.1 Hz (H-5).

MS (rel int.): m/z 456 (M^+,30), 335 (15), 307 (20), 295 (100), 279 (6), 239 (20), 121 (8) and 107 (12).

The reaction of 2-amino-3-formylchromone (50) with triacetic acid lactone (61)

Formation of 3-acetoacetyl-5-oxo-5H-[1]benzopyrano [3, 2-e] pyridin-2-one (62)

2-Amino-3-formylchromone (1.0 g, 5.29 mmol) was dissolved in pyridine (30 ml) containing piperidine (0.89 ml, 10.5 mmol) and triacetic acid lactone (0.999 g,7.936 mmol) added to it. The reaction mixture was kept at room temperature for 7 days. The product 3-acetoacetyl-5-oxo-5H-[1]benzopyrano [3, 2-e] pyridin-2-one **62** crystallised out as yellow solid. It was filtered, washed with cold water and dried. The mother liquor was poured in to ice-cold water and acidified with HCl. The solid, which precipitated out was filtered, washed with water, dried and recrystallised from chloroform-methanol to afford more **62**.

M.P. 180°C.

Yield 1.3g, 82%;

Spectral Data:

UV (MeOH) λ_{max}: 263.76, 299.08, 396.33 nm.

IR (KBr)ν_{max}: 1652, 1647, 1642, cm^{-1};

^{1}HNMR (300MHz, DMSO):

δ2.28 s (CH_3), 7.09 s (H_a), 8.2 d, J=9Hz (C-6); 8.85 s, (H_b), 14.0 s (OH, D_2O exchangeable), 16.3 s, (OH, D_2O exchangeable).

MS:(rel Int.): m/z 297 (M^+62%), 282 (6), 254(11), 240(4), 212(3), 169(4), 159(16), 157 (100), 141(6), 121(6).

The reaction of 2-amino-3-formylchromone (50) with triacetic acid lactone (61)

Formation of 6-methyl-2-(4-oxo-4H-1-benzopyran-3-yl)-3-(2'-hydroxybenzoyl)-4-pyridone (68).

1.0 g(5.29 mmol) of 2-amino-3-formylchromone was dissolved in 20 ml of alcohol and potassium acetate 0.519 g (5.29 mmol) and triacetic acid lactone 0.99g (7.93 mmol) added to it. The resultant mixture was refluxed on water bath for 12 hours. It was concentrated and allowed to stand at room temperature when light yellow solid crystallized out from the reaction mixture. It was filtered, washed with alcohol and dried. The compound was recrystallized from chloroform and methanol to afford 6-methyl-2-(4-oxo-4H-1-benzopyran-3-yl)-3-(2'-hydroxybenzoyl)-4-pyridone **68** as yellow shining crystals.

M.P. : 215-220°C

Yield : 1.38 g

Spectral Data:

UV(MeOH)λ_{max}: 242.37,261.11,296.48,370.62 nm.

IR: 3398, 3249, 1650, 1616, 1620, 1508, 1463, 1432, 1331 and 760 cm^{-1}.

^1H NMR (DMSO d_6):

δ 213 s, 3H (CH_3), 7.07 s, 1H (H-5), 7.31-7.70 m, 6H (Ar-H), 8.02 d, 1H, J=9Hz (H-5'), 8.61 s, 1H (H-2'), 9.58 br s, 1H (NH) and 16.68 br s, 1H (OH).

MS (rel. Int.): m/z 374 (M^++1, 48), 345 (39), 336 (83), 298(36), 289(22), 283 (14), 256 (8), 254 (3), 240 (17), 228 (17), 222 (6), 211 (6), 192 (100), 190 (20), 166 (3), 154 (61), 120 (6), 121 (4), 107 (11), 91 (8), 89 (11) and 76 (8).

The reaction of 2-amino-3-formylchromone (50) with 5, 5-dimethyl-cyclohexane-1,3-dione:

Formation of 3,3-dimethyl-5-oxo-cyclohexa[2,3-b] azaxanthone (78):

2-Amino-3-formylchromone 1 g (5.29 mmol) was dissolved in 40 ml of pyridine containing 0.899 ml (10.5 mmol) of piperidine and added 5, 5-dimethylcyclohexane-1, 3-dione, 0.740 gm (5.29 mmol) to it. The reaction mixture was allowed to stand at room temperature for 5 days. The solid as obtained, was filtered, washed with water, dried and recrystalised from chloroform to afford 3,3-dimethyl-5-oxo-cyclohexa[2,3-b] azaxanthone **78** as yellow shining crystals.

M.P. 250^{0}C

Yield 1.4gm, 90%

Spectral Data:

UV (MeOH) λ_{max}: 261.11, 331.90 nm.

IR (KBr)ν_{max}: 3429, 1685, 1651, 1596, 1472, 1403, 1317 and 768cm^{-1}.

^{1}H NMR ($CDCl_3$): δ 1.17 s, 6H (2 x CH_3), 2.64 s, 2H (CH_2), 3.14 s, 2H ($COCH_2$), 7.47-7.83 m, 3H (Ar-H), 8.33 1H, d, J=7.8 Hz, (H8), 9.27 s, 1H.

MS (rel. int.): m/z 294 (M^++1, 100), 278 (17), 276 (6), 262 (6), 238 (6), 211 (6), 202 (3), 180 (3), 120 (3) and 115 (4).

The reaction of 2-amino-3-formylchromone 50 with 3-methyl-1-phenyl-5-pyrazolone.

Formation of methylidene-bis-4,4-(3-methyl-5-oxo-1-phenylpyrazole) (81):

2-Amino-3-formylchromone 1.0g (5.29 mmol) was dissolved in 20 ml of dry methanol, added 5 drops of piperidine and 0.922g (5.29 mmol) of 3-methyl-1-phenyl-5-pyrazolone to it. The resultant mixture was refluxed on water bath for 2 hours. It was allowed to cool at room temperature. The reaction mixture was poured into ice-cold water and acidified with HCl. The solid methylidene-bis-4.4- (3-methyl-5-oxo-1-phenylpyrazole) **81** which precipitated out was filtered, washed with water, dried and recrystallized from chloroform-methanol as yellow crystals. The filtrate was extracted with chloroform, washed with water, dried over anhydrous sodium sulfate and evaporated. The solid as obtained was recrystalized from chloroform-methanol to get more methylidene-bis-4, 4-(3-methyl-5-oxo-1-phenylpyrazole **81**.

M.P. – 150-160^{0}C

Yield – 1.52 gm

Spectral Data:

UV (MeOH)λ_{max}: 240.38, 406 nm.

IR (KBr): 3350, 1627, 1592, 1550, 1498 and 1328 cm^{-1};

^{1}HNMR ($CDCl_3$): δ 2.32 s, 6H (CH_3), 7.26-7.92 m, 11H (Ar-H + methylene proton).

MS (rel. int.): m/z 358 (M^+, 100), 357 (50), 340 (5), 281(6), 117 (4), 104 (4), 90 (20) and 77(35).

Piper cubeba

Extraction and isolation

The seed of the plant were collected from Mysore, India. The plant material was further identified in the Department of Botany, AMU, Aligarh (India). The seeds of *Piepr cubeba* (5 Kg) were air dried under shade and powdered. The powdered material was exhaustively extracted with petrol and then with benzene at room temperature for 15 days.

Both petrol and benzene extracts were concentrated under reduced pressure to afford gummy residues (30g). The TLC of both the extracts displayed same type of spots. The two extracts were, therefore, combined and subjected to column chromatography, using benzene ethyl acetate as eluant. The compound isolated was labeled as KCB.

KCB:

It was obtained using benzene-ethyl acetate (75.25, v/v) as eluant and crystallized from benzene to yield white crystalline solid. It was identified as cubebin **100** through comparison with authentic sample (m.p., IR, NMR and mass).

MP: 120-22°C

Yield: 3.5g

Spectral Data:

UV (MeOH)λ_{max}: 239.74, 287.20 nm.

IR (KBr)ν_{max}: 3418, 1629, 1491 and 926 cm^{-1}.

^{1}H NMR (DMSO):δ 6.61-6.81 m 6H, 5.95 s, 4H (O-CH_2-O), 5.03 t, 1H (C-1 proton), 3.81-3.86 m, 2H (-OCH_2), 3.35 s, 1H (OH) and 1.82-2.27 m, 6 H.

^{13}C NMR (300 MHz, CDCl3):

δ 103.2 (C-2), 53.0 (C-3), 45.7 (C-4), 72.5 (C-5), 39.1 (C-6), 38.7 (C-7), 100.7 (O-CH_2O), 132.2 (C-1"), 108.7 (C-2"), 147.9 (C-3"), 145.6 (C-4"), 109.1 (C-5"), 121.6 (C-6"), 133.8 (C-1'), 109.0 (C-2'), 147.4 (C-3'), 145.7 (C-4'), 109.0 (C-5'), 121.2 (C-6').

MS (rel. int.): m/z 356 (M^+, 42), 339 (17), 209 (10), 203 (13), 136 (100), 135 (87), 122 (8).

Zanthoxylum simularis

Extraction and Isolation:

The plant was collected from Nepal Border and a sample of it was identified by the Department of Botany, Tribhuvan University of Nepal. The stems wood (15 kg) was chapped into small piece and shade dried. It was defatted with petroleum ether and extracted with chloroform in cold for 15 days. The solvent was removed under reduced pressure to furnish a brown residue (30 g). It was adsorbed on silica gel (60-120 mesh) and subjected to column chromatography using chloroform–petrol (60-80) as eluant. The compounds isolated were labelled as ZS-1 and ZS-2

ZS-1

Elution of the column with chroform-methanol petrol (50:50, v/v) gave a fraction which on evaporation afforded a yellowish mass (5 g). The yellowish residue was further chromatographed over silica gel using chloroform-petrol (70:30, v/v) as eluent. The appropriate fractions were combined and evaporated to give a solid which on crystallization from chloroform yielded **ZS-1**. It was characterized as limonin through direct comparison with (IR, ^{1}H NMR, ^{13}C NMR and mass) with an authentic sample.

M .P. : 275°C

Yield: 100 mg

Spectral Data:

IR (Nujol) ν_{max}: 1755, 1710, 1460, 1380, 1160, 920, 880 and 875 cm^{-1}.

1H NMR (300 MHz, DMSO):

δ 1.007 (C-8 Me), 1.03 (C-18Me) 1.11, 1.19 (C-4-Methyls), 1.23-1.88 (4H, m, C-10, C-11 CH_2), 2.25-2.3 (1H, dd, H-9, J=14.76, 3.2 Hz), 2.58-2.66 (1H, dd, H-2 J=16.59, 3.9 Hz), 3.07-3.17 (1H, t, H-1), 3.31 (1H, m, H-5), 4.11 (1H, s, H-15), 4.50 (1H, d, H-19, J=13.02 Hz), 4.94 1H, d, H-19 J=13.05 Hz), 5.47 (1H, s, H-17), 6.15 (1H, m, H-21), 7.66 (1H, m , H-20). 7.72, (1H, m, H-23).

^{13}C NMR (300 MHz):

δ 78.2 (C-1), 35.39 (C-2), 169.67 (C-3), 79.24 (C-4), 57.9 (C-5), 35.9(C-6), 207.58(C-7), 50.15 (C-8), 46.3(C-9), 50.15(C-10) 17.36(C-11), 28.9(C-12),

37.5(C-13), 66.49(C-14), 53.59(C-15), 166.6(C-16), 77.24 (C-17), 0.0 (C-18), 64.6(C-19), 120.04 (C-20), 141.4 (C-21), 109.9(C-22), 143.04 (C23).

MS (rel. int.): m/z 413 (7), 348 (23), 347 (100), 329 (15), 287 (8), 241 (5), 227 (5), 145 (10), 136 (13), 135 (30), 121 (18), 108 (28), 95 (50), 91 (25) and 69 (27).

Further elution of the extract with chloroform afforded 4',5-dihydroxy-6", 6"-dimethylpyrano (2", 3", 7, 6) flavone **106**.

M.P.: 295-98°C

Yield: 60 mg

IR (Nujol): 3400, 1650, 1600, 1450, 1350, 1240, 1180, 1130 and 840 cm^{-1}

^1H NMR (60 MHz, $CDCl_3$ + DMSO d_6):

δ 1.5 (6 H, s, CH_3), 5.6 (1H, d, J=~10 Hz), 6.2 (1H, s, H-8), 6.5 (1H, s, H-3), 6.70-7.55 (3H, m, Olefinic H, H-3', H-5'), 7.65 -7.85 (2H, d, H-2', H-6').

MS (rel. int.): m/z 336 (M^+, 52), 322 (40), 321 (100), 203 (60), 159 (10), 118 (5).

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